Reversible pulmonary trunk banding: VII. Stress echocardiographic assessment of rapid ventricular hypertrophy in young goats

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Background: Ventricle retraining with abrupt systolic overload can cause myocardial edema and necrosis, followed by late ventricular failure. Intermittent systolic overload could minimize the inadequacy of conventional pulmonary artery banding. The present study compared ventricle function under dobutamine stress in 2 protocols of systolic overload in young goats.

Methods: Nineteen young goats were divided into 3 groups: sham (n = 7; no systolic pressure overload), continuous (n = 6; systolic overload maintained for 96 hours), and intermittent (n = 6; 4 periods of 12-hour systolic overload, paired with a 12-hour resting period). Echocardiographic and hemodynamic evaluations were performed daily. The myocardial performance index and ejection fraction were evaluated at rest and during dobutamine stress. The goats were then killed for morphologic evaluation.

Results: The intermittent group underwent less systolic overload than the continuous group ($P < .05$). Nevertheless, both groups had increased right ventricular and septal masses compared with the sham group ($P < .0002$). Echocardiography revealed a major increase in right ventricular wall thickness in the intermittent group (+64.8% ± 23.37%) compared with the continuous group (+43.9% ± 19.26%; $P = .015$). Only the continuous group remained with significant right ventricular dilation throughout the protocol ($P < .001$). The intermittent group had a significantly better myocardial performance index at the end of the protocol, under resting and dobutamine infusion, compared with the continuous group ($P < .012$).

Conclusions: Both systolic overload protocols have induced rapid right ventricular hypertrophy. However, only the intermittent group had better preservation of right ventricular function at the end of the protocol, both at rest and during dobutamine infusion. (J Thorac Cardiovasc Surg 2013;145:1345-51)

Supplemental material is available online.

In developing countries, the number of patients with transposition of the great arteries (TGA) presenting beyond the neonatal period is still considerable. In this scenario, complete anatomic repair must be preceded by left ventricle retraining with pulmonary artery banding, to enable the ventricle to handle the systemic circulation. Concern is increasing about the quality of subpulmonary ventricle retraining, aiming at the most physiologic hypertrophic process to avoid late ventricular dysfunction.[1]

Previous studies have looked for better alternatives to subpulmonary ventricle retraining. It has been demonstrated in a young animal model that intermittent systolic overload, in agreement with athletic philosophy, has promoted more prominent hypertrophy, compared with continuous systolic overload.[2,3] However, these studies assessed global right ventricular (RV) systolic function only at rest, without showing any significant differences between the systolic overload protocols.

Nevertheless, the evaluation of RV function is difficult to image because of its complex morphology. Although cardiac magnetic resonance imaging is currently considered the reference technique for RV volumetry and calculation of the ejection fraction, various echocardiographic parameters can provide reliable information on RV dimensions and RV systolic and diastolic function in daily clinical practice.[4] Therefore, the myocardial performance index (MPI) has been proposed as a relatively simple method to assess the combined systolic and diastolic performance of the right ventricle simultaneously, at rest and under dobutamine stress.[5]

The present study aimed at a more detailed echocardiographic assessment of subpulmonary ventricular function in 2 different protocols of systolic overload, using MPI and a pharmacologic stress technique.

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Abbreviations and Acronyms

LV = left ventricular
MPI = myocardial performance index
PAB = pulmonary artery banding
PT = pulmonary trunk
RV = right ventricular
RVEDV = RV end-diastolic volume
RVEF = RV ejection fraction
RVMPI = RV myocardial performance index
TGA = transposition of the great arteries

METHODS

Nineteen young goats, aged 30 to 60 days were enrolled in the present study and divided into 3 groups of comparable weight ($P = .84$): sham (n = 7; weight, 12.00 ± 2.65 kg), continuous (n = 6; weight, 11.27 ± 3.20 kg), and intermittent (n = 6; weight, 11.98 ± 1.07 kg). All goats received humane care in accordance with the guidelines established by the Brazilian College of Animal Experimentation. The ethics committee for research protocols at the University of São Paulo School of Medicine reviewed and approved the present study (CAPPesq 0664/09).

ANESTHESIA

Anesthesia was induced with intramuscular ketamine (20 mg/kg) and maintained with ketamine (1 mg/kg intravenously) and nembutal (5 mg/kg intravenously) on demand. The goats were intubated and mechanically ventilated (Harvard 708, South Natick, Mass). An electrocardiogram was recorded and blood pressure measurements were taken with computer software (ACQknowledge, version 3.01; Biopac Systems, Inc, Goleta, Calif). Antibiotic therapy (cephazolin 500 mg intravenously and gentamicin 40 mg intramuscularly) was administered just before the operation and maintained during the protocol. Digoxin (0.01 mg/kg) and heparin (5000 IU) were also administered throughout the protocol. All the goats were extubated right after the surgical procedure and remained ambulatory and breathing spontaneously throughout the protocol.

SURGICAL PROCEDURE

The chest was opened at the fourth left intercostal space to expose the RV outflow tract, after lung retraction. A 17-gauge heparinized catheter was inserted in the RV outflow tract, pulmonary trunk (PT), and descending aorta for pressure measurements at specific intervals during the entire study. The adjustable pulmonary artery banding (PAB) system (SILIMED; Silicone e Instrumental Medico-Cirurgico e Hospitalar Ltda, Rio de Janeiro, Brazil) was implanted just beyond the pulmonary valve, as previously described.[6]

RV SYSTOLIC OVERLOAD PROTOCOL

RV training was begun after a 72-hour convalescence period with percutaneous PAB insufflation with saline solution to achieve an RV/systemic pressure ratio of 0.7, limited by a 10% decrease in systolic blood pressure. Readjustments were made every morning throughout the protocol. If systemic hypotension and/or respiratory distress developed after PAB inflation, it was deflated to a volume compatible with RV tolerance and maintenance of goat hemodynamics. A 96-hour study period has been previously established as the minimum time required for cardiac masses equalization in young goats.[7]

Continuous Group Protocol

In the continuous group, the goats remained with continuous systolic overload for 96 hours, with daily assessment to keep the RV/aortic pressure ratio at 0.7. Hemodynamic data were collected once daily (mornings) during PAB readjustments.

Intermittent Group Protocol

In the intermittent group, the goats underwent 4 daytime periods of 12-hour systolic overload, alternating with a 12-hour nighttime resting period. Hemodynamic data were collected every 12 hours, during PAB readjustments.

Sham Group Protocol

In the sham group, the PAB system was maintained deflated during the entire protocol. Hemodynamic data were collected once daily (mornings).

ECHOCARDIOGRAPHY

All the goats while under light sedation (ketamine 15 mg intramuscularly) were examined by a single experienced observer preoperatively and daily throughout the study and monitored continuously with surface electrocardiography. The following echocardiographic parameters were studied using multifrequency transducers (7.5 and 2.5 MHz, Acuson Cypress; Siemens, Erlagen, Germany): left ventricular (LV), RV, and ventricular septum wall thicknesses, RV end-diastolic volume (RVEDV) and RV end-systolic volume, RV end-diastolic diameter, RV ejection fraction (RVEF), and RV MPI (RVMPI).

Because of the keel structure of the goat thorax, the RV free wall thickness was taken from the parasternal long-axis 4-chamber view and from the parasternal short-axis view (at basal and papillary muscle levels), where the limits of the RV free wall were more easily obtained. The LV end-diastolic posterior and septal wall thicknesses were measured through the parasternal long-axis view, at the level of the mitral valve leaflet tips. All the wall thicknesses were measured using 2-dimensional technique, under resting conditions.

Also, from the long-axis, 4-chamber view, the RVEF was measured by the modified Simpson rule. The RVMPI was calculated from pulsed wave Doppler of RV inflow and outflow tracts, positioning the sample volume at the level of the tricuspid and pulmonary valve leaflet tips, respectively, as previously described by Ishii and colleagues.[8] This index

expresses the ratio between isovolumic times (contraction and relaxation) and represents the assessment of RV systolic and diastolic function. It was obtained at rest and during pharmacologic stress with dobutamine, according to the following formula: $(IVCT + IVRT) \times ET^{-1}$, where IVCT represents the isovolumetric contraction time; IVRT, the isovolumetric relaxation time; and ET, the ejection time. The values were obtained through the long-axis, 4-chamber view, using the Doppler method, by positioning the volume sample at the center of the tricuspid valve and measuring the interval between the onset of valve closure and the beginning of the next diastole (time a).

The RV ejection time was calculated with the volume sample located in the RV outflow tract (time b) through the parasternal short-axis view. The value of isovolumetric contraction time + isovolumetric relaxation time was obtained by subtracting time a from time b. This result was then divided by the ejection time, providing the RVMPI value. The mean of 3 consecutive measurements were then obtained to achieve the final RVMPI value for each goat.

The assessment of RV function under dobutamine infusion was performed during 2 periods: before the surgical procedure (baseline) and at protocol end (96 hours). During the stress echocardiographic examination, a graded dobutamine infusion was started at 5 μg/kg/min and increased at 3-minute intervals to 10, 20, 30, and 40 μg/kg/min.[9] The endpoints were the achievement of a target heart rate, defined as 1.7 of the baseline heart rate. When the target heart rate was achieved, the echocardiographic parameters RVEF and RVMPI were measured.

MORPHOLOGIC ASSESSMENT

The goats were humanely killed after 96 hours of the study protocol. After harvesting the heart, the pericardial fat, both atria, and semilunar valves were dissected from it. The right ventricle, left ventricle, and ventricular septum were separated using the Fulton technique, individually weighed (METTLER AE-200; Mettler-Toledo AG, Greifensee, Switzerland), and indexed to each goat's body weight. The water content was obtained individually in each cardiac chamber by subtracting the collected sample weight at necropsy from the weight of the dehydrated chamber (70 hours at 60°C). Values were obtained as a percentage of weight change.

STATISTICAL ANALYSIS

The data are presented as the mean ± standard deviation. A comparison of variables was performed with 2-way analysis of variance, except for body weight, wall masses, and water content, which were performed with 1-way analysis of variance. Both analyses were followed by the Bonferroni post hoc test. The systolic overload imposed on the right ventricle of the continuous and intermittent groups was assessed by calculating the areas under the curve (trapezoidal method). The comparison between these areas was performed using the unpaired Student *t*-test. Statistical analysis was performed using GraphPad Prism, version 4 (GraphPad Prism, La Jolla, Calif) and SigmaStat, version 3.11.0 (Systat Software, Inc, Chicago, Ill).

RESULTS

Hemodynamic Measurements

The systolic RV-PT gradient increased in both study groups ($P < .001$) and in every measurement period ($P < .001$). It was greater than that in the sham group, from the beginning of RV systolic overload (0 hour) until the end of the protocol ($P < .001$). The area under the RV-PT gradient curve was calculated, as a method of measuring the RV systolic overload imposed on each group. The systolic overload of the continuous group (4597 ± 814 mm Hg × h) and intermittent group (2777 ± 384.6 mm Hg × h) was greater than that in the sham group (480 ± 101.10 mm Hg × h). The continuous group was the most exposed to systolic overload ($P < .05$). Parallel to the RV-PT gradient, both study groups showed an increased RV/aorta systolic pressure ratio over time (Table E1) compared with its respective baseline value and with that in the sham group at the same point ($P < .001$).

Echocardiographic Findings at Rest

Both continuous and intermittent groups had an increased RV free wall thickness compared with the preoperative measurements and the sham group at 72 and 96 hours ($P < .001$). However, the RV thickness increase was significantly greater in the intermittent group at the end of the protocol (5.38 ± 0.82 mm) compared with that in the continuous (4.65 ± 0.48 mm) and sham (3.36 ± 0.08 mm) groups ($P = .001$). No differences were seen in the septum and LV thicknesses among the 3 groups ($P = .10$) or throughout the protocol ($P > .82$). The graph showing the RV free wall thickness changes throughout the protocol is available on-line (Figure E1).

RV end-diastolic volume changes were significantly different between the 2 groups throughout the protocol ($P < .001$). Both study groups showed a significant increase in RVEDV immediately after the onset of RV systolic overload (intermittent, 112.33% ± 87.24%, $P = .002$; and continuous, 98.3% ± 123.5%, $P = .007$). At 24 hours, the intermittent group had recovered RVEDV to a value comparable to that in the sham group, but in the continuous group, the RVEDV continued to be significantly increased at 24 (101.1% ± 70.3%, $P = .03$), 72 (87.6% ± 87.3%, $P = .03$), and 96 (92.7% ± 89.9%, $P = .015$) hours compared with the sham group. The graph showing the RV end-diastolic volume changes is available on-line (Figure E2).

The RVEF changes throughout the study period were significantly different between groups ($P < .001$) and over time ($P < .001$). Both study groups had significant RVEF

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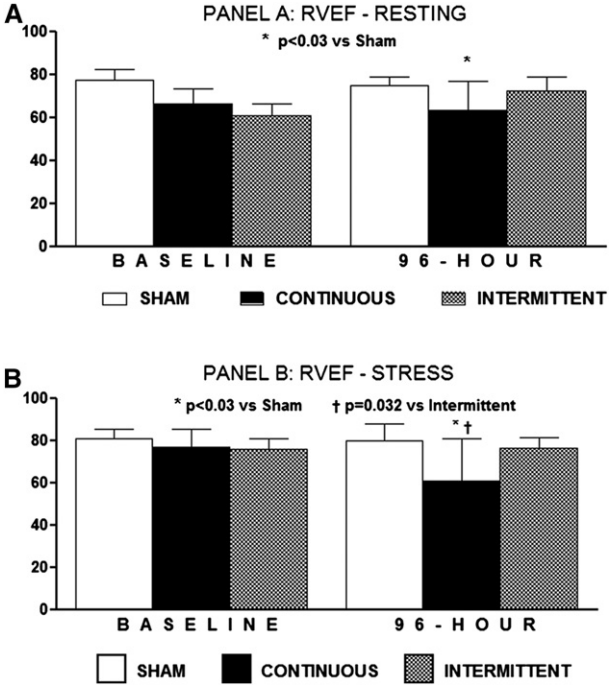

FIGURE 1. Right ventricular ejection fraction *(RVEF)* of sham, continuous, and intermittent groups, under dobutamine stress. A, Resting condition (*$P < .03$ compared with that in sham group at measurement point) and, B, dobutamine stress (*$P < .03$ compared with that in sham group at measurement point). †$P = .032$ compared with that in intermittent group at measurement point.

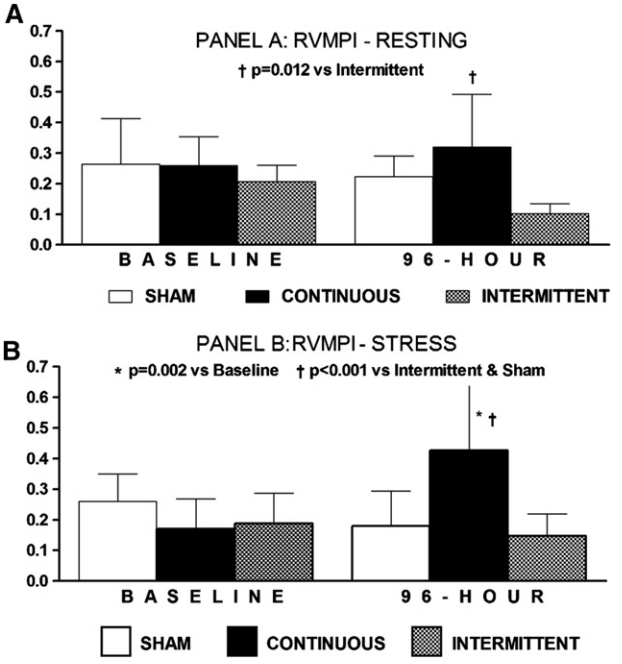

FIGURE 2. Right ventricular myocardial performance index *(RVMPI)* of sham, continuous, and intermittent groups, under dobutamine stress. A, Resting condition (†$P = .012$ compared with that in intermittent group at measurement point) and, B, dobutamine stress (*$P = .002$ compared with baseline value; †$P < .001$ compared with that in intermittent and sham groups at measurement point).

impairment immediately after the onset of RV systolic overload (intermittent, –47.7% ± 26.9%, $P = .001$; and continuous, –39.6% ± 27.8%, $P = .002$). Subsequently, the intermittent-group RVEF recovered to values comparable to those in the sham group, and the continuous group maintained prolonged RVEF impairment at 24 (–38.8% ± 23.1%, $P < .001$) and 48 (–19.6% ± 32.4%, $P = .029$) hours compared with the sham group. After 72 hours of systolic overload, the RVEF in both study groups had recovered to values comparable to those in the sham group. The RVEF data of the 3 groups throughout the study period are shown on line in Table E2 and Figure E3.

In contrast to the RVEF findings, the RVMPI values in the continuous group were worse after 72 hours (0.38 ± 0.17) and 96 hours (0.41 ± 0.28) of systolic overload, with a significant difference from that in the sham group at 72 hours (0.16 ± 0.08; $P = .009$) and from both the sham (0.22 ± 0.07; $P = .039$) and intermittent (0.10 ± 0.03; $P < .001$) groups at 96 hours. These data are also available on-line (Table E3 and Figure E4).

Echocardiographic Findings Under Pharmacologic Stress

Dobutamine infusion caused a similar heart rate increase in the 3 groups, at both baseline ($P = .66$) and the end of the protocol ($P = .10$). The heart rate data are available on-line (Table E4).

Figure 1 shows the RVEF before surgical intervention (baseline) and at the end of the protocol, at rest (Figure 1, *A*) and under pharmacologic stress (Figure 1, *B*). Although the 3 groups completed the protocol with a normal RVEF at rest, the continuous group had the smallest RVEF value (63.00% ± 13.90%) compared with that in the sham group (74.71% ± 4.19%; $P = .026$). It was observed that the RVEF in the intermittent group had improved after the ventricular training period (72.33% ± 6.28%), relative to its baseline condition (60.83% ± 5.34%; $P = .013$). During the dobutamine stress test, the RVEF in the 3 groups remained within the normal range. However, the continuous group again had the lowest RVEF (60.67% ± 20.30%) compared with that in the sham (79.57% ± 8.02%; $P = .026$) and intermittent (76.33% ± 4.97%; $P = .032$) groups.

The RVMPI graph of the 3 groups during baseline and at 96 hours, at rest and under dobutamine stress is shown in Figure 2. The baseline values were similar among the 3 groups, with or without dobutamine. However, the continuous group had unfavorable RVMPI rates at the end of the protocol, both at rest (0.32 ± 0.17) and with dobutamine stress (0.43 ± 0.21) compared with the intermittent group during rest (0.10 ± 0.03; $P < .012$) and under

TABLE 1. Cardiac mass weight of sham, continuous, and intermittent groups, indexed to goat body weight

Cardiac mass (g/kg)	Sham (n = 7)	Continuous (n = 6)	Intermittent (n = 6)
Right ventricle	0.76 ± 0.12	1.21 ± 0.11*	1.15 ± 0.07*
Septum	0.86 ± 0.10	1.18 ± 0.12*	1.04 ± 0.10*
Left ventricle	1.38 ± 0.17	1.45 ± 0.19	1.53 ± 0.27

Data presented as mean ± standard deviation. *$P < .05$ compared with sham group.

pharmacologic stress (0.15 ± 0.07; $P < .012$). Compared with the sham group during dobutamine infusion (RVMPI, 0.18 ± 0.11), the RVMPI of the continuous group increased significantly ($P < .001$).

Morphologic Findings

Cardiac mass weight. Both continuous and intermittent systolic overload resulted in significant increases in RV (intermittent, 51.3%; continuous, 59.2%; $P < .05$) and septal masses (intermittent, 20.9%; continuous, 37.2%; $P = .05$) compared with those in the sham group (Table 1). No significant changes were noted in LV mass ($P = .4662$).

Myocardial water content. A minor, although significant, increase was seen in the water content of the RV myocardium of the continuous (+3.2%) and intermittent (+3.0%) groups ($P = .0102$) compared with that in the sham group. Similarly, increased water content was observed in the septum of the continuous (+2.9%) and intermittent (+2.4%) groups ($P = .0028$) compared with that in the sham group. These data are available on-line in Table E5.

DISCUSSION

In the present study, we aimed to assess subpulmonary ventricular function using 2 pulmonary trunk banding protocols, focusing on a more sensitive method to detect early disturbances in ventricular function during the process of rapid RV hypertrophy. In addition to Simpson's method, we used the MPI to evaluate ventricular function with a greater sensitivity and accuracy, especially in goats, which usually have poor imaging windows because of their keel-shaped chest. The combination of RVMPI calculation, which did not require morphologic analysis of the heart chamber or the perfect visualization of endocardial borders, with stress echocardiography resulted in significant improvement in ventricular function assessment in this rapid ventricular hypertrophy protocol. It was clearly demonstrated that intermittent systolic overload, although quantitatively smaller than continuous overload, has promoted functionally superior hypertrophy under dobutamine stress at the 96-hour study period. Even at rest, the intermittent group had a significantly more favorable RVMPI than the continuous group had after the training period. However, both study groups evolved with important RVEF impairment immediately after starting the protocol, owing to acute systolic overload of 70% systemic pressure, which resulted in RV systolic dysfunction earlier in the protocol. In contrast to the RVMPI results, the initial RVEF worsening recovered by the end of the protocol in both study groups, to values comparable to those in the sham group, with or without dobutamine infusion. Therefore, systolic dysfunction might have occurred in both study groups at the beginning, with diastolic dysfunction only in the continuous group at the end of the protocol. RVMPI worsening might have been caused by a diastolic disorder, although it is not possible to quantify isolated systolic or diastolic index changes. At the end of protocol, the difference between the 2 echocardiographic methods was more evident with the MPI, which should indicate a greater sensitivity for assessing ventricular function and enhance diagnostic confidence in ventricular retraining.

Regarding the RV end diastolic volume, continuous systolic overload resulted in persistent dilation throughout the protocol. This could also reflect worse ventricular adaptation in the continuous group. However, the 12-hour resting periods might have optimized subendocardial coronary flow in the intermittent group and, consequently, replenished energy substrates to the myocardial hypertrophy process, limiting the severity of systolic overload. That would probably provide a better adaptation and consequent preservation of ventricular function in the intermittent group at periods of systolic overload.

MPI has been used in a wide variety of heart diseases in adults and children, including congenital univentricular heart lesions and in the follow-up of cavopulmonary connections.[10] Its use has recently been reported in fetal echocardiography.[11] Previous studies from Tei and colleagues[12] have demonstrated the usefulness of the MPI in the evaluation of systolic and diastolic dysfunction in patients with idiopathic dilated cardiomyopathy. In aortic stenosis, MPI is used to assess both the severity of aortic stenosis and the presence of LV contractile reserve.[13] It can provide important prognostic information in patients with associated LV systolic dysfunction, because surgical treatment with aortic valve replacement appears to improve the outcome for most patients with LV contractile reserve. In contrast, surgery is associated with high mortality in the absence of a contractile reserve. Similarly, we can derive the same MPI capacity to differentiate goats with RV systolic overload, artificially induced by PAB, among those with or without preserved ventricular function.

The results of the present study might reflect the morphologic findings of Carroll and colleagues[14] in pigs that underwent continuous systolic overload for 7 hours. They found intense inflammatory infiltrate in the myocardium, with foci of cellular necrosis in varying degrees and subsequent late ventricular dysfunction, probably related to unbalanced oxygen demand in the hypertrophic myocardium. Evidence has shown that abrupt systolic overload can evolve, with

systolic dysfunction associated with diastolic disorder or just isolated filling restriction, as demonstrated by Leeuwenburgh and colleagues.[15] They have demonstrated decreased cardiac output in young sheep that underwent PAB, despite the better RV contractility. This divergence is probably related to diastolic dysfunction, by increased RV relaxation time and decreased ventricular compliance. Therefore, the assessment of diastolic function should ideally be a part of the echocardiographic examination during subpulmonary ventricle retraining.

Morphologic Versus Echocardiographic Assessment of Hypertrophy

According to the echocardiographic analysis, the intermittent group had a greater RV free wall thickness than the continuous group, despite less exposure to systolic overload. These data were not supported by the morphologic analysis. Both protocols promoted an increase in septal and RV masses of similar magnitude in the study groups. Perhaps, the greater RV dilation observed by echocardiography in the goats in the continuous group influenced the interpretation of the RV free wall thickness. Regarding myocardial water content, the mild increase observed in the septum and right ventricle in both trained groups would not justify the increased myocardial mass weight, suggesting that it was probably related to increased protein synthesis and cell proliferation triggered by systolic overload and not to cellular and/or interstitial edema, as previously documented by Abduch and colleagues.[16]

Clinical Implications

The preparation of the subpulmonary ventricle, required to perform the 2-stage Jatene operation beyond the neonatal period, is still considered an alternative approach in several pediatric cardiology centers worldwide. According to Kang and colleagues,[17] late diagnosis of congenital heart disease is remarkable in several countries and up to 95% of infants remain untreated. However, the echocardiographic, hemodynamic, and clinical criteria in decision making for the optimal surgical treatment of TGA beyond the neonatal period are still controversial. The presence of LV collapse on echocardiography, a ventricular mass index less than 35 g/m^2, LV/systemic blood pressure ratio less than 0.67, and age older than 3 weeks are parameters suggested by several investigators to select candidates for ventricular retraining before anatomic repair.[18,19] In addition to the controversy about those parameters and the quality of myocardial tissue hypertrophy, the indication for the 2-stage operation has been debated. Several centers have reported satisfactory results with primary anatomic repair in unfavorable cases using mechanical circulatory support devices or extracorporeal membrane oxygenation.[20] Nevertheless, the obtained results have still been difficult to reproduce in other centers. Notwithstanding the uncertainties, with the current development of the ventricular retraining technique and because of the unavailability of the routine use of extracorporeal membrane oxygenation or ventricular assist devices in some cardiac centers, and the RV late dysfunction occurring after the Mustard and Senning operations, LV retraining remains as an important option for the 2-stage Jatene operation in selected cases. As mentioned previously, the high morbidity and mortality rates observed with traditional PAB are probably related to the intense and abrupt continuous pressure overload and can cause LV dysfunction, neoaortic regurgitation, and RV outflow tract obstruction. Therefore, intermittent systolic overload to prepare the subpulmonary ventricle, simulating the physical conditioning of athletes, might provide a better customization of ventricular retraining and improve its effects on myocardial function. Accordingly, a more physiologic hypertrophy might be achieved, aiming at preservation of future ventricular function. The results of the present study suggest that intermittent systolic overload can improve clinical management during LV retraining interstage for patients with TGA beyond the neonatal period and in those with congenitally corrected TGA or after atrial baffle operations with RV failure. It could favorably affect the results for a 2-stage Jatene operation.

Study Limitations

Considering the limitations of clinical inferences from observations of experimental studies, we cannot be certain sure that the behavior of the human left ventricle with TGA would act exactly the same as observed in the right ventricle of young goats. Although the architecture of the cardiomyocytes in the RV walls is comparable to that found in the left ventricle in terms of endocardial and epicardial helical angles, the right ventricle in both the normal and the hypertrophied state lacks the extensive zone of circular myocytes seen in the midportion of the LV walls. This architectural arrangement in a circular fashion can generate greater cavity pressures, with important implications in the systolic and diastolic functions of the left ventricle.[21] Another important concern is that the continuous systolic overload imposed on the right ventricle was adjustable and not created with a traditional fixed band, commonly used in clinical practice. In the present study, the systolic overload might be less acute and intense using a percutaneously adjustable PAB than when using traditional PAB, in which no changes are possible during ventricular retraining. Nevertheless, the coronary arterial circulation is not submitted to a hypertensive regimen, which is more closely related to the rapid 2-stage approach.[16] Regarding the MPI, it is important to emphasize that no reference values have been established for goats. Even in children and neonates, a disparity exists between the normal values.[22] In the present study, the baseline values were taken as a reference for subsequent analysis during the systolic overload protocol.

Previous studies have shown that in the absence of an increased ventricular preload or myocardial infarction, there is a good correlation between the values measured by the MPI using conventional Doppler and tissue Doppler, which was not possible in these animals because of the absence of an apical 4-chamber view.[23,24]

CONCLUSIONS

The results of the present study have demonstrated that both systolic overload protocols promoted ventricular hypertrophy that was more evident in the intermittent group on echocardiography, despite less exposure to RV systolic overload. There was preservation of systolic and diastolic function in the intermittent group, both at rest and during dobutamine stress. In contrast, the functional performance of the continuous group was worse during rest and pharmacologic stress. The use of MPI in conjunction with stress echocardiography improved the accuracy of the diagnosis in assessment of dysfunction during subpulmonary ventricle retraining.

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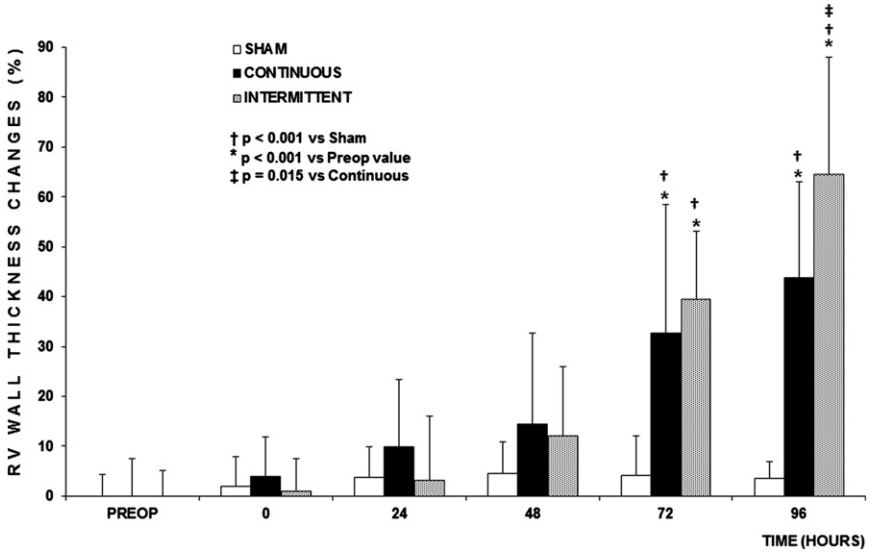

FIGURE E1. Right ventricular *(RV)* thickness changes in sham, continuous, and intermittent groups throughout protocol. †$P < .001$ compared with that of sham group; *$P < .001$ compared with respective baseline value; and ‡$P = .015$ compared with that in continuous group at same measurement point.

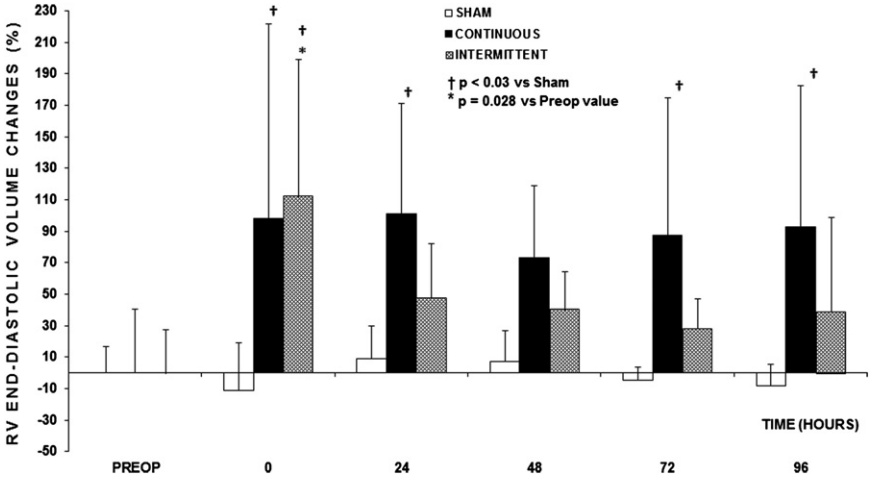

FIGURE E2. Right ventricular *(RV)* end-diastolic volume changes in sham, continuous, and intermittent groups throughout protocol. †$P < .03$ compared with that of sham group; *$P = .028$ compared with its respective preoperative *(Preop)* value.

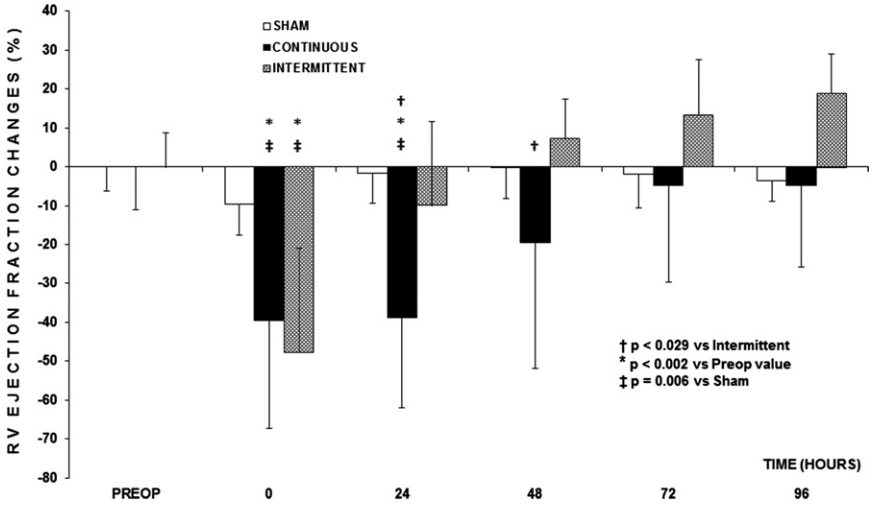

FIGURE E3. Right ventricular *(RV)* ejection fraction changes (%) of sham, continuous, and intermittent groups throughout protocol, under resting conditions. †$P < .029$ compared with that in intermittent group at same measurement point; *$P < .002$ compared with its respective preoperative *(Preop)* value; and ‡$P = .006$ compared with that in sham group at same measurement point.

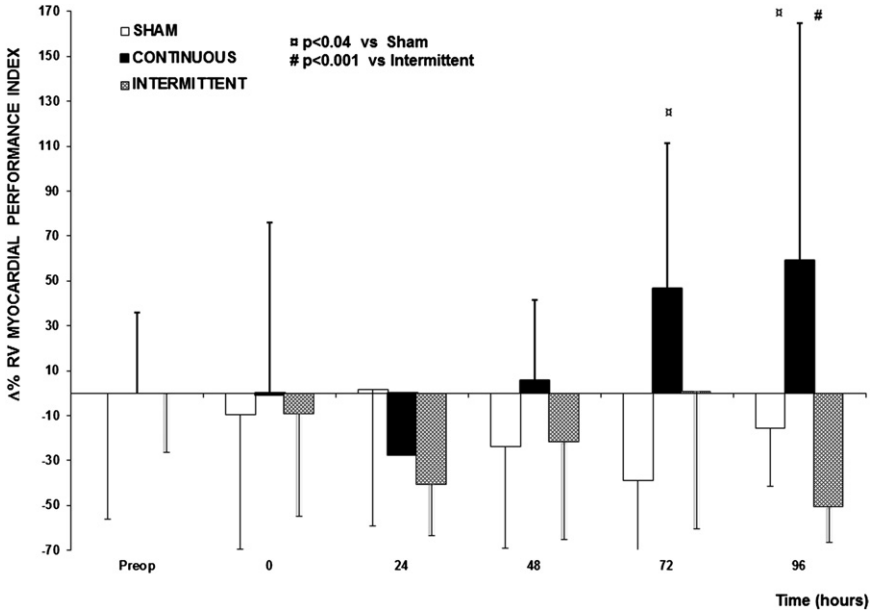

FIGURE E4. Right ventricular *(RV)* myocardial performance index changes (%) of sham, continuous, and intermittent groups throughout protocol, under resting conditions. ¤$P < .04$ compared with that in sham group at same measurement point; and #$P < .001$ compared with that in intermittent group at same measurement point.

TABLE E1. RV/aorta systolic pressure ratio of sham, continuous, and intermittent groups throughout protocol

Measurement point (h)	Sham (n = 7)	Continuous (n = 6)	Intermittent (n = 6)
Baseline	0.36 ± 0.06	0.33 ± 0.05	0.32 ± 0.05
0	0.36 ± 0.06	0.66 ± 0.08*	0.70 ± 0.06*
24	0.34 ± 0.06	0.72 ± 0.10*	0.75 ± 0.05*
48	0.33 ± 0.08	0.78 ± 0.14*	0.78 ± 0.09*
72	0.38 ± 0.10	0.93 ± 0.30*	0.81 ± 0.15*
96	0.28 ± 0.06	0.71 ± 0.23*	0.81 ± 0.12*

Data presented as mean ± standard deviation. *RV*, Right ventricular. *$P < .001$ compared with sham group at same measurement point and respective baseline value.

TABLE E2. RVEF of sham, continuous, and intermittent groups throughout protocol

Measurement point (h)	Sham (n = 7)	Continuous (n = 6)	Intermittent (n = 6)
Preoperative	77.43 ± 4.89	66.17 ± 7.28	60.83 ± 5.34*
0	70.00 ± 6.27	40.00 ± 18.38*,†	31.83 ± 16.35*,†
24	76.14 ± 5.90	40.50 ± 15.27*,†	54.83 ± 13.20*
48	77.29 ± 6.24	53.20 ± 21.41*	65.33 ± 6.19
72	76.00 ± 6.78	63.00 ± 16.47	69.00 ± 8.65
96	74.71 ± 4.19	63.00 ± 13.90	72.33 ± 6.28

Data presented as ± standard deviation. *RVEF*, Right ventricular ejection fraction. *$P < .02$ compared with sham group for same measurement point. †$P < .001$ compared with preoperative value.

TABLE E3. RVMPI of sham, continuous, and intermittent groups throughout protocol

Measurement point (h)	Sham (n = 7)	Continuous (n = 6)	Intermittent (n = 6)
Preoperative	0.26 ± 0.15	0.26 ± 0.09	0.21 ± 0.05
0	0.24 ± 0.16	0.26 ± 0.20	0.19 ± 0.09
24	0.27 ± 0.16	0.19 ± 0.03	0.12 ± 0.05
48	0.20 ± 0.12	0.28 ± 0.09	0.16 ± 0.09
72	0.16 ± 0.08	0.38 ± 0.17*	0.21 ± 0.13
96	0.22 ± 0.07	0.41 ± 0.28*,†	0.10 ± 0.03

Data presented as mean ± standard deviation. *RVMPI*, Right ventricular myocardial performance index. *$P < .039$ compared with sham group for same measurement point. †$P < .001$ compared with intermittent group for same measurement point.

ET/BS

TABLE E4. Heart rate of sham, continuous, and intermittent groups at rest and under pharmacologic stress, with respective doses of dobutamine reached by each group

Measurement point	Group	HR at rest (bpm)	Dobutamine dose (μg/kg/min)	Δ% HR	HR with stress (bpm)
Baseline					
	Sham	134.86 ± 23.36	31.43 ± 9.00	60.99 ± 20.21	206.29 ± 48.22
	Continuous	131.50 ± 16.88	36.67 ± 8.16	59.79 ± 21.05	210.33 ± 39.70
	Intermittent	126.00 ± 12.70	30.00 ± 12.60	84.69 ± 30.50	229.83 ± 17.50
96-h					
	Sham	114.86 ± 22.93	30.71 ± 13.67	61.81 ± 16.11	184.86 ± 36.10
	Continuous	131.83 ± 14.62	35.00 ± 8.37	56.16 ± 29.88	204.67 ± 36.30
	Intermittent	130.00 ± 16.94	24.17 ± 13.57	72.20 ± 7.00	223.33 ± 24.68

Baseline, $P = .66$ between groups; 96-h, $P = .10$ between groups. *HR*, Heart rate; *HR with stress*, heart rate during dobutamine infusion.

TABLE E5. Water content of cardiac masses of sham, continuous, and intermittent groups

Cardiac mass (g/kg)	Sham (n = 6)	Continuous	Intermittent	*P* value
Right ventricle	78.84 ± 2.41	82.00 ± 1.11*	81.85 ± 1.41*	.0102
Septum	77.11 ± 2.08	80.04 ± 0.27*	79.53 ± 0.75*	.0028
Left ventricle	78.20 ± 1.89	79.23 ± 0.40	79.08 ± 0.69	.2887

Data presented as mean ± standard deviation. *$P < .01$ compared with sham group.

Biventricular structural and functional responses to aortic constriction in a rabbit model of chronic right ventricular pressure overload

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Objectives: Chronic right ventricular (RV) pressure overload results in pathologic RV hypertrophy and diminished RV function. Although aortic constriction has been shown to improve systolic function in acute RV failure, its effect on RV responses to chronic pressure overload is unknown.

Methods: Adjustable vascular banding devices were placed on the main pulmonary artery and descending aorta. In 5 animals (sham group), neither band was inflated. In 9 animals (PAB group), only the pulmonary arterial band was inflated, with adjustments on a weekly basis to generate systemic or suprasystemic RV pressure at 28 days. In 9 animals, both pulmonary arterial and aortic devices were inflated (PAB+AO group), the pulmonary arterial band as for the PAB group and the aortic band adjusted to increase proximal systolic blood pressure by approximately 20 mm Hg. Effects on the functional performance were assessed 5 weeks after surgery by conductance catheters, followed by histologic and molecular assessment.

Results: Contractile performance was significantly improved in the PAB+AO group versus the PAB group for both ventricles. Relative to sham-operated animals, both banding groups showed significant differences in myocardial histologic and molecular responses. Relative to the PAB group, the PAB+AO group showed significantly decreased RV cardiomyocyte diameter, decreased RV collagen content, and reduced RV expression of endothelin receptor type B, matrix metalloproteinase 9, and transforming growth factor β genes.

Conclusions: Aortic constriction in an experimental model of chronic RV pressure overload not only resulted in improved biventricular systolic function but also improved myocardial remodeling. These data suggest that chronically increased left ventricular afterload leads to a more physiologically hypertrophic response in the pressure-overloaded RV. (J Thorac Cardiovasc Surg 2012;144:1494-501)

The right ventricle (RV) may be subjected to an abnormally high afterload in patients with various types of congenital and acquired heart disease and also in idiopathic, hereditary or other forms of pulmonary arterial (PA) hypertension.[1] The RV typically shows progressive remodeling, including increased wall thickness, myocardial hypertrophy, and interstitial fibrosis. This initially adaptive compensatory hypertrophy eventually becomes maladaptive and may eventually progress to RV dilatation and failure,[2,3] the development of which is a universally poor prognostic feature.[4] Maladaptive hypertrophy and interstitial fibrosis are usually associated with marked changes in myocardial fibrotic signaling. Among others increased levels of endothelin 1 (ET-1), matrix metalloproteinase (MMP) 9, and transforming growth factor β (TGF-β) have been observed as markers of maladaptive hypertrophy and fibrosis in models of left ventricular (LV) dysfunction and failure and may also play a role in RV failure.[5]

The pathophysiology of RV failure, particularly with regard to adverse ventricular-ventricular interactions, is becoming increasingly understood. For example, in a study of patients with chronic idiopathic pulmonary hypertension, right heart dilation altered LV geometry and diastolic function, as assessed by Doppler echocardiography, and was associated with worse outcomes.[6] Furthermore, in a magnetic resonance study, Gan and coworkers[7] demonstrated that cardiac output was inversely related to LV end-diastolic dimension (because it is compressed by the RV), rather than to RV function per se.[7] Conversely, it may therefore be possible that modification of these adverse ventricular-ventricular interactions may lead to some benefits. It is well known that superficial myocardial fibers are shared and continuous between the RV and the LV, providing an anatomic basis for normal and abnormal ventricular-ventricular interactions.[8] Indeed, in an elegant

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Abbreviations and Acronyms

CTGF = connective tissue growth factor gene
EDN1 = endothelin 1 gene
EDNRA = endothelin receptor type A gene
EDNRB = endothelin receptor type B gene
EDPVR = end-diastolic pressure-volume relationship
ESPVR = end-systolic pressure-volume relationship
ET-1 = endothelin 1
GAPDH = glyceraldehyde 3-phosphate dehydrogenase gene
LV = left ventricle
MMP = matrix metalloproteinases
MMP2 = matrix metalloproteinase 2 gene
MMP9 = matrix metalloproteinase 9 gene
MT = Masson trichrome
PA = pulmonary artery
PRSW = preload recruitable stroke work
PSR = picrosirius red
RV = right ventricle
TGF-β = transforming growth factor β

study of normal hearts, in which the ventricles were electrically isolated but mechanically intact, Damiano and colleagues[9] showed that under basal conditions LV contraction contributed more than 65% of the work of the normal RV. These normal interactions form the basis of beneficial interventions in models of acute RV failure. Yamashita and coworkers[10] showed that increased LV afterload, produced by aortic constriction, both restored LV dimensions and improved RV stroke volume in the setting of acute pulmonary embolic shock. Similarly, Belenkie and coworkers[11] showed a beneficial effect of aortic constriction (independent of changes in coronary flow) in experimental RV failure induced by acute banding of the PA,[11] and our own recent data in a similar PA-banding model showed that improvement in RV contractility assessed by conductance catheter could be induced both by acute aortic banding and by pharmacologically increasing LV afterload with norepinephrine.[12] We speculated that the improved RV contractility was manifested through harnessing of the Anrep effect of increased LV afterload, leading to increased LV contractility, which in turn was transmitted to the RV through the crosstalk mechanism shown by Damiano and colleagues,[9] but clearly other factors, such as changes in septal position, may also contribute to this phenomenon.

To date, there have been no studies of the effect of increased LV afterload on RV responses in chronic RV failure. The purpose of this study was therefore to investigate the effect of chronic aortic constriction on biventricular structural and functional responses in an experimental model of chronic RV pressure overload. We hypothesized that aortic constriction would improve RV function and myocardial remodeling in this setting.

MATERIALS AND METHODS

All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the article as written. All experiments were approved by the animal ethics committee of the Hospital for Sick Children, Toronto, Ontario, Canada, and were done in accordance with the "Guiding Principles in the Care and Use of Animals" of the American Physiologic Society.

Preparation

Twenty-three adult New Zealand white rabbits were studied. After intravenous cannulation (24G Angiocath; BD, Franklin Lakes, NJ) of the ear marginal vein, anesthesia was initiated by the use of isoflurane (3%) and acepromazine maleate (INN acepromazine, 1.1 mg/kg). After tracheal intubation, ventilation was controlled mechanically to maintain normal blood gas values ($Paco_2$ of 32-35 mm Hg). General anesthesia was maintained with isoflurane (1.5%-2%). Heart rate and oxygen saturation were continuously monitored.

Banding Devices

For incremental banding of the pulmonary trunk and ascending aorta, we used a recently developed adjustable banding device (ABS; Silimed Inc, Rio de Janeiro, Brazil).[13] The band consists of a banding ring, a connecting tube, and an inflation reservoir. The banding ring is a C-shaped hydraulic cuff with a 5 mm width and a rigid outer layer, reinforced with a polyester mesh, which keeps it from deforming centrifugally. The cuff compresses the lumen of the vessel when expanded in proportion to the volume injected into the inflation reservoir. The connecting tube hermetically connects the banding ring to the inflation reservoir, which is covered by a silicone membrane to allow repeated percutaneous needle puncture for adjustment of the degree of vascular constriction. In all animals, adjustable banding devices were placed on the pulmonary trunk and descending aorta through a left lateral thoracotomy, then the connecting tubes were tunneled toward each of the inflation reservoirs, which were placed subcutaneously in the neck.

After band placement, the animals were then allocated to either the PAB group, which underwent PA band inflation alone (n = 9); the PAB+AO group, which underwent both PA and aortic band inflation (n = 9); or the sham group, which did not have the devices inflated (n = 5).

After a recovery period of 7 days, each animal in the PAB and PAB+AO groups received stepwise percutaneous inflation of the cuff around the PA to induce RV pressure overload. Doppler echocardiography was used to monitor RV pressure (estimated by Doppler assessment of the tricuspid regurgitation) and the RV/PA pressure gradient (estimated by Doppler assessment of gradient across the PA band). Three incremental PA band adjustments were performed on a weekly basis. Initially, the banding was calibrated to an RV pressure of approximately half systemic pressure, at second banding to between two thirds and three quarters of systemic pressure, and to systemic levels on the 3rd occasion. In the PAB+AO group, the aortic banding was also adjusted at each stage to maintain a gradient of approximately 20 mm Hg across the band (as measured by Doppler echocardiography).

Functional Assessment

Hemodynamic measurements were performed in all animals 5 weeks after pulmonary banding-operation (after 2 weeks of systemic RV pressure). A 3F conductance catheter (Millar Instruments, Inc, Houston, Tex) was advanced to each ventricle for simultaneous measurement of pressures and

volumes through a neck vein and a neck artery, respectively. For preload reduction, required to obtain pressure-volume relationships, a balloon catheter (10-mm Tyshak balloon; NuMED Canada Inc, Cornwall, Ontario, Canada) was introduced through a groin vein and was placed in the inferior caval vein under fluoroscopic guidance. For all conditions, steady-state hemodynamic data were recorded during short periods of suspended ventilation at end-expiration. Indices of systolic and diastolic function were derived from pressure-volume loops recorded during inflation of a balloon catheter in the inferior caval vein to reduce RV preload. For systolic ventricular function, we determined end-systolic elastance as the slope of the end-systolic pressure-volume relationship (ESPVR) and preload recruitable stroke work (PRSW) from a family of pressure-volume loops during progressive occlusion of the inferior caval vein. Diastolic stiffness was determined as the slope of the end-diastolic pressure-volume relationship (EDPVR).

Tissue Collection

Each animal was killed after hemodynamic measurements were made. The LV and RV samples were cut into 2 parts, 1 of which was immediately snap-frozen in liquid nitrogen and stored at –80°C for RNA and protein analysis. The other part was fixed in 10% neutral-buffered formaldehyde and embedded in paraffin. The 5-μm cross-sections of RV and LV were cut and stained with hematoxylin-eosin, picrosirius red (PSR) F3BA, and Masson trichrome (MT) stains.

Histologic Analysis

Interstitial collagen in transverse cardiac section was evaluated by PSR and MT staining. Interstitial collagen was identified in the PSR-stained sections by its red appearance and in the MT-stained sections by its blue appearance. The cardiac interstitial collagen content was measured and expressed as a percentage of the total collagenous and noncollagenous areas in the entire visual field of the section by automated planimetry with Adobe Photoshop CS2 software (Adobe Systems Inc, San Jose, Calif). The myocyte diameter was measured with images captured from hematoxylin-eosin–stained sections. A point-to-point perpendicular line was placed across the longitudinally cut myocyte at the level of the nucleus, and this diameter length was then measured by the National Institutes of Health ImageJ analysis program (http://rsbweb.nih.gov/ij/). All the longitudinally directed myocytes with a distinct cell border (at the level of the nucleus) within the sampling field were measured and averaged to provide the mean cardiomyocyte diameter. Transverse or obliquely cut myocytes were excluded. Cell width was determined from longitudinally positioned myocytes to reduce the error of determining such for myocytes that might not have been cut precisely perpendicular to their long axis.

Real-Time Reverse Transcriptase–Polymerase Chain Reaction Analysis

Total RNA was extracted from RV and LV tissues with TRIzol Reagent (Invitrogen Corp, Grand Island, NY). Reverse transcription proceeded with 1 μg of total RNA with SuperScript III First-Strand Synthesis System (Invitrogen). Real-time polymerase chain reaction was carried out with SYBR Green Master Mix (Applied Biosystems, Life Technologies Corporation, Carlsbad, Calif). Glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used as the endogenous reference. Primers are listed as follows: ET-1 gene (*EDN1*), forward 5′-ACTTCTGCCACCTGGACATCA-3′, reverse 5′-ACGCTGCCCTGGTAGGAAAT-3′; endothelin receptor type A gene (*EDNRA*), forward 5′-GCTTCTTGCTGCTCATGGATTAC-3′, reverse 5′- CCGAGGTCATCAGGCTCTTG-3′; endothelin receptor type B gene (*EDNRB*), forward 5′-CTGGCCATTTGGAGCTGAGA-3′, reverse 5′-TTTGGAACCCCAATTCCTTTAA-3′; MMP-2 gene (*MMP2*), forward 5′- AGGACTACGACCGCGACAAG-3′, reverse 5′- TGTTGCCCAG GAAGGTGAAG -3′; MMP-9 gene (*MMP9*), forward 5′-CTTCCAACTTT GACAGCGACA-3′, reverse 5′-GGAGTGATCCAAGCCCAGTG-3′; TGF-β gene (*TGFB*), forward 5′-AGGGCTACCACGCCAACTT-3′, reverse: 5′-CCGGGTTGTGCTGGTTGTAC-3′, connective tissue growth factor gene (*CTGF*), forward 5′-CCCTGCGTCTTCGGTGGC-3′, reverse 5′-AGGCAGTTGGCTCGCATCAT-3′; and *GAPDH*, reverse 5′-AGG CCGTGGGCAAGGT-3′, reverse 5′-CCTCGGATGCCTGCTTCA-3′.

Statistical Analysis

Data are expressed as mean ± SEM. Results were analyzed by analysis of variance for repeated measurements. GraphPad software (GraphPad Software Inc, San Diego, Calif) was used for statistical analysis.

RESULTS

Hemodynamic and Functional Characteristics

During the postsurgical periods, all animals gained weight and showed no clinical signs of heart failure. Water and food were available ad libitum, and intake was normal. Populations were similar in each group in terms of age and body weight at time of the initial and terminal experiments. Hemodynamic and functional data are listed in Table 1. As expected, PA banding resulted in a significant increase in RV systolic pressure relative to the sham group ($P < .01$) and was not different between the PAB and PAB+AO groups. The slopes of RV ESPVR and RV PRSW were also significantly increased in

TABLE 1. Hemodynamic and functional data

	Sham	PAB	*P* value*	PAB+AO	*P* value†	*P* value‡
RV Pes (mm Hg)	14.4 ± 1.2	52.6 ± 3.1	<.01	52.7 ± 2.3	<.01	NS
RV Ped (mm Hg)	1.6 ± 0.4	2.1 ± 0.4	NS	4.0 ± 0.7	<.01	<.05
RV PRSW (mm Hg)	1.5 ± 1.3	4.1 ± 0.9	<.05	13.6 ± 5.1	<.0001	<.05
RV ESPVR (mm Hg/mL)	2.7 ± 0.3	7.7 ± 2.9	<.05	13.9 ± 4.9	<.0001	<.05
RV EDPVR (mm Hg/mL)	0.8 ± 0.3	1.3 ± 0.5	NS	2.2 ± 0.5	NS	NS
LV Pes (mm Hg)	36.1 ± 2.2	48.0 ± 0.9	NS	74.3 ± 3.9	<.01	<.05
LV Ped (mm Hg)	1.2 ± 0.5	1.5 ± 0.5	NS	4.9 ± 0.6	<.01	<.01
LV PRSW (mm Hg)	13.3 ± 5.7	13.4 ± 1.2	NS	22.6 ± 4.6	<.05	<.05
LV ESPVR (mm Hg/mL)	10.6 ± 0.9	11.3 ± 2.5	NS	18.2 ± 1.5	<.05	<.05
LV EDPVR (mm Hg/mL)	1.1 ± 0.1	1.3 ± 0.3	NS	2.4 ± 0.4	<.05	<.05

All values are mean ± SEM. *EDPVR*, End-diastolic pressure-volume relationship; *ESPVR*, end-systolic pressure-volume relationship; *LV*, left ventricular; *NS*, not significant ($P > .05$); *PAB*, pulmonary arterial banding alone [group]; *PAB+AO*, pulmonary arterial and aortic banding [group]; *Pes*, end-systolic pressure; *Ped*, end-diastolic pressure; *PRSW*, preload recruitable stroke work; *RV*, right ventricular. *Sham versus pulmonary arterial banding alone. †Sham versus pulmonary arterial banding plus aortic banding. ‡Pulmonary arterial banding alone versus pulmonary arterial banding plus aortic banding.

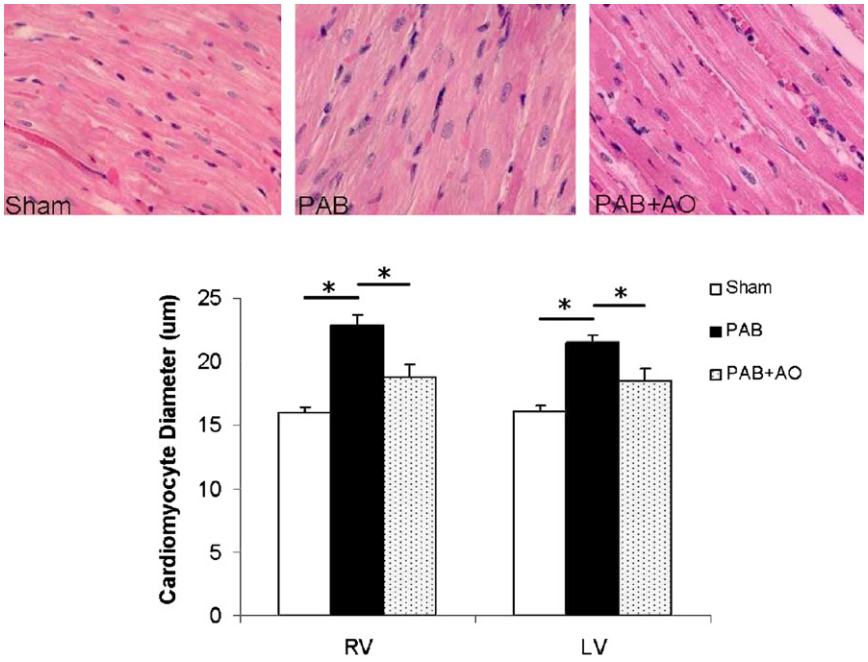

FIGURE 1. Hematoxylin-eosin staining showed a significantly increased cardiomyocyte diameter in the pulmonary arterial banding only (*PAB*) group for the right ventricle (*RV*) and for the left ventricle (*LV*). Addition of aortic banding (*PAB+AO*) resulted in a significantly decreased cardiomyocyte diameter for both ventricles. *Asterisk* indicates $P < .05$.

the PAB group relative to the sham group. End-diastolic pressure in the RV tended to increase in the PAB group relative to the sham group, but not significantly. In the LV, end-systolic pressure, end-diastolic pressure, and the slopes of LV ESPVR and LV PRSW did not differ significantly between the PAB group and the sham group.

Additional aortic banding (PAB+AO) resulted in further functional improvement. Relative to the PAB group, ESPVR and PRSW were significantly higher in the PAB+AO group for the RV (RV ESPVR, 13.9 vs 7.7 mm Hg/mL; $P < .05$; RV PRSW, 13.6 vs 4.1; $P < .05$) and for the LV (LV ESPVR 18.2 vs 11.3 mm Hg/mL; $P < .05$; LV PRSW, 22.6 vs 13.4; $P < .05$), reflecting improved biventricular contractility.

Diastolic RV stiffness, as assessed by RV EDPVR, was not different in either intervention group relative to the sham group, whereas the end-diastolic RV pressure was significantly increased in the PAB+AO group relative to the PAB and sham groups. In the LV, there were significant increases in both LV EDPVR and end-diastolic LV pressure in the PAB+AO group relative to the PAB and sham groups.

Histologic Remodeling

Hematoxylin-eosin staining showed significantly increased cardiomyocyte diameters in the PAB group relative to the sham group (22.88 ± 0.87 μm vs 16.03 ± 0.47 μm for the RV and 21.53 ± 0.54 μm vs $16.12 \pm 0.41$$\mu$m for the LV; Figure 1). Additional aortic banding resulted in significantly decreased cardiomyocyte diameters in the PAB+AO group (18.8 ± 0.99 μm for the RV and 18.47 ± 1.01 μm for the LV) relative to the PAB group.

Both PSR and MT staining revealed significant increases in collagen in both the RV (PSR staining, $10.78\% \pm 1.39\%$ vs $3.74\% \pm 0.34\%$; MT staining, $9.2\% \pm 1.73\%$ vs $2.12\% \pm 0.31\%$) and the LV (PSR staining, $5.28\% \pm 0.48\%$ vs $2.86\% \pm 0.3\%$; MT staining, $2.5\% \pm 0.46\%$ vs $0.9\% \pm 0.23\%$) for the PAB group relative to the sham group ($P < .05$). Addition of aortic banding resulted in significant decreases in collagen in both the RV (PSR staining, $6.61\% \pm 0.85\%$; MT staining, $4.78\% \pm 0.77\%$) and the LV (PSR staining, $3.81\% \pm 0.36\%$; MT staining, $1.8\% \pm 0.33\%$) for the PAB+AO group ($P < .05$) (Figure 2).

Molecular Assessment

Expression of *EDN1* was significantly increased in the RV myocardium in both the PAB group (2.22 ± 0.42) and the PAB+AO group (2.18 ± 0.28) relative to the sham group (1.07 ± 0.19; $P < .05$; Figure 3, *A*). Interestingly, expression of *EDN1* was also significantly increased in the LV myocardium in both the PAB group (2.5 ± 0.26) and the PAB+AO group (2.99 ± 0.19) relative to the sham group (1.14 ± 0.26; $P < .05$; Figure 4, *A*). RV and LV myocardial expressions of *EDNRA* were not different in either intervention group relative to the sham group (RV, 1.16 ± 0.11 and 1.06 ± 0.07 vs 1.02 ± 0.11, respectively; *P* not significant), (LV, 1.1 ± 0.06 and 0.89 ± 0.07 vs 1.02 ± 0.11,

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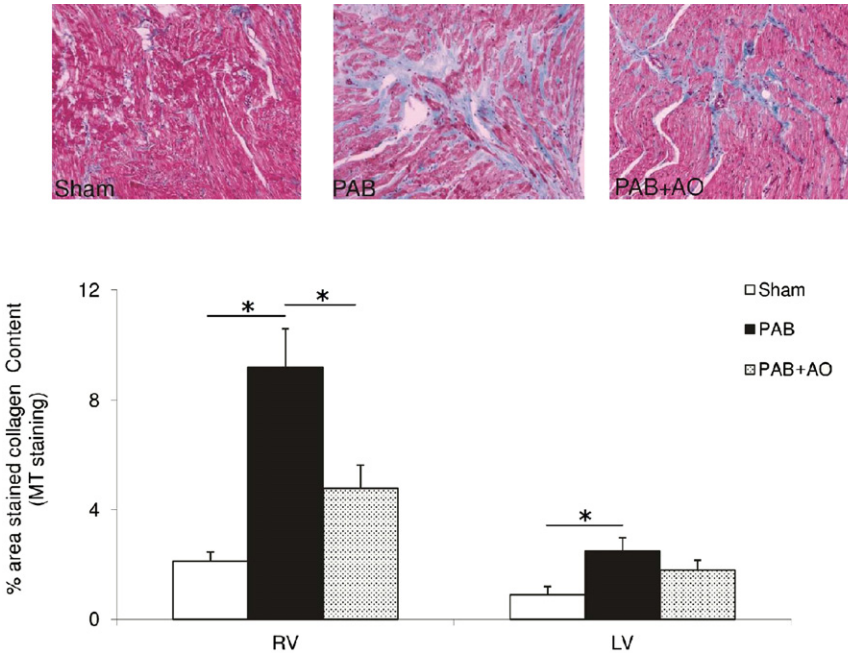

FIGURE 2. Representative sections showing Masson trichrome (*MT*) staining for collagen content. The bar graph of the quantitative analysis shows increased collagen in response to pulmonary arterial banding (*PAB*) in both the right ventricle (*RV*) and the left ventricle (*LV*). Addition of aortic banding (*PAB+AO*) significantly attenuated this increase in the right ventricle but not in the left ventricle. *Asterisk* indicates $P < .05$.

respectively; *P* not significant). *EDNRB* expression, in contrast was significantly elevated in the RV in the PAB group (1.95 ± 0.3) relative to the sham group (1.02 ± 0.1; $P < .05$) but not in the PAB+AO group (1.52 ± 0.3; *P* not significant; Figure 3, *A*). There were no differences among the groups in *EDNRB* expression in the LV (Figure 4, *A*).

MMP9 expressions were significantly elevated in the RV myocardium in both the PAB group (2.12 ± 0.32) and the PAB+AO group (1.38 ± 0.12) relative to the sham group (0.88 ± 0.2; $P < .05$), whereas in the LV myocardium *MMP9* expressions were not different in either the PAB group (2.32 ± 0.39) or the PAB+AO group (1.83 ± 0.18) relative to the sham group (1.21 ± 0.43; *P* not significant). *MMP2* expressions were not elevated (1.07 ± 0.16 and 0.96 ± 0.13 vs 1.01 ± 0.16) in either ventricle. In the RV myocardium, TGF-β expression was significantly increased in the PAB group (1.4 ± 0.13) relative to the sham group (0.84 ± 0.16; $P < .05$; Figure 3, *B*) and was significantly lower in the PAB+AO-group (0.93 ± 0.1) relative to the PAB group ($P < .05$). A similar pattern of TGF-β expression was seen in the LV (Figure 4, *B*). There were no statistically significant differences in *CTGF* expressions between any of the groups in either ventricle.

DISCUSSION

This study demonstrates that chronic aortic constriction not only improves RV function but also leads to less maladaptive RV remodeling in response to chronic RV pressure overload.

Progressive RV remodeling, including increased wall thickness and myocardial hypertrophy, as well as interstitial fibrosis, is a hallmark of PA hypertension.[14] This initially adaptive hypertrophy eventually becomes maladaptive, and RV failure ensues. Clinically manifest RV failure is consistently reported as an adverse risk factor for survival in patients with pulmonary hypertension,[4] and although its onset may be delayed by the use of advanced pulmonary vasodilator therapies, such as prostanoids, phosphodiesterase type 5 inhibitors, and endothelin receptor blockers, in the absence of a curative therapy it is likely that progression to RV failure and death will be the final common pathway for these patients. Although not ignoring the huge impact of vasodilator therapies, the potential for modifying RV function as a method of improving symptoms and survival has been emphasized in several recent reviews.[15-17] Whereas these reviews have speculated that specific therapies may be developed to target the RV itself, there is evidence from both experimental and clinical studies that symptoms and outcomes may be driven by adverse ventricular-ventricular interactions related to the dilated and poorly functioning RV modifying the systolic and diastolic performance of the LV. We believe that the heart failure associated with progressive pulmonary hypertension should be considered a biventricular disease and that therapies targeted toward both ventricles may therefore be beneficial. Indeed, we and others have shown that other ventricular-ventricular interactions can be harnessed for potential therapeutic benefit, at least acutely, in the setting of pulmonary hypertension.[11,12] For

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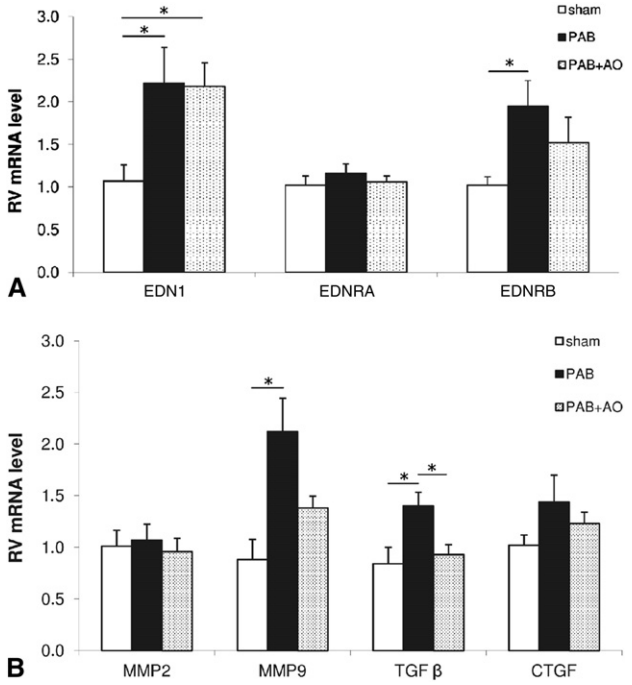

FIGURE 3. Results of real-time polymerase chain reaction analysis of right ventricular (*RV*) myocardium. A, Expressions of endothelin 1 gene (*EDN1*) and endothelin receptor B gene (*EDNRB*) expressions were significantly increased in the pulmonary arterial banding (*PAB*) group and in the group with added aortic banding (*PAB+AO*) relative to the sham group, while there was no effect on endothelin receptor A gene (*EDNRA*) expression. B, Messenger RNA (*mRNA*) levels for genes for matrix metalloproteinases 2 (*MMP2*) and 9 (*MMP9*), transforming growth factor-β (*TGF* β) and connective tissue growth factor (*CTGF*). Expressions of matrix metalloproteinase 9 and transforming growth factor β genes were significantly increased in the pulmonary arterial banding group relative to the sham group. Addition of aortic banding was associated with significantly reduced transforming growth factor β gene expression and a trend toward reduced matrix metalloproteinase 9 gene expression. There was no statistically significant change in connective tissue growth factor gene expression. *Asterisk* indicates $P < .05$.

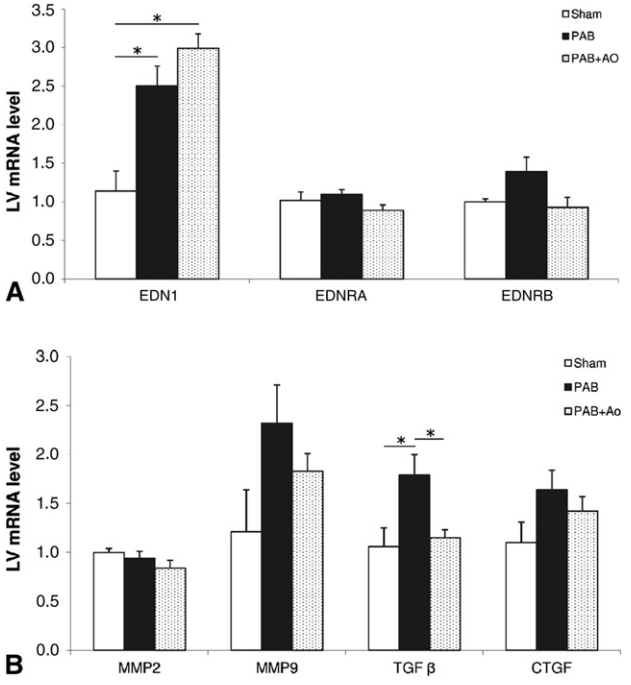

FIGURE 4. Results of real-time polymerase chain reaction analysis of left ventricular (*LV*) myocardium. A, Expression of endothelin 1 gene (*EDN1*) was significantly increased in left ventricular myocardium in the pulmonary arterial banding (*PAB*) group and in the group with added aortic banding (*PAB+AO*) relative to the sham group, while there was no effect on endothelin receptor A gene (*EDNRA*) and endothelin receptor B gene (*EDNRB*) expressions. B, Messenger RNA (*mRNA*) levels for genes for matrix metalloproteinases 2 (*MMP2*) and 9 (*MMP9*), transforming growth factor β (*TGF* β), and connective tissue growth factor (*CTGF*). Transforming growth factor β gene was significantly increased in the left ventricular myocardium in the pulmonary arterial banding group relative to the sham group. Addition of aortic banding was associated with significantly reduced transforming growth factor β gene expression. There was no statistically significant change in matrix metalloproteinase 2, matrix metalloproteinase 9, and connective tissue growth factor gene expressions in the left ventricular myocardium. *Asterisk* indicates $P < .05$.

example, increasing LV contractility through the Anrep effect (increased LV contractility in response to increased LV afterload by aortic constriction or pharmacologic systemic arterial vasoconstriction) leads to improved RV contractility in models of acute RV failure. To date, there have been no studies of this potential effect on biventricular responses to a chronically increased RV afterload, despite the potential therapeutic benefits that this novel approach may offer.

In the chronic PA banding model used in this study, RV remodeling was characterized by increases in both RV mass and cardiomyocyte diameter. In addition, there was excessive interstitial myocardial fibrosis, as evidenced by increased collagen content, characteristic of the failing RV myocardium.[14] Concomitant aortic banding not only partially abrogated these histologic changes but also was associated with significantly improved RV function. Indeed, both the RV and the LV showed significantly improved systolic function, as measured by relatively load-independent indices derived from conductance catheter measurements. It is important to note that these improvements occurred in the presence of maladaptive RV remodeling (RV hypertrophy, increased cardiomyocyte diameter, and fibrosis) but before the development of overt RV failure (preserved RV ESPVR). When extrapolating to potential clinical applications, this seems to be the window when intervention would be most timely—when signs of RV remodeling are present, but before overt and irreversible RV failure has occurred.

The maladaptive hypertrophy seen in the PAB group was associated with marked changes in myocardial fibrotic signaling. The profile of increased *EDN1*, *MMP9*, and TGF-β expressions observed in the PAB group is consistent with the

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maladaptive hypertrophy and fibrosis described in models of LV dysfunction and failure, and each presents potential therapeutic targets identified in a recent comprehensive review.[5] Our data are consistent with those shown for the pressure-loaded LV, and the effect of aortic banding is consistent with it being an intervention that alters the maladaptive signature, toward a more physiologic response. Concomitant aortic banding led to a decrease in histologic fibrosis and was associated with reduced TGF-β, *CTGF*, and *MMP9* expressions.

Although the signaling pathways that relate to these molecules have been studied extensively in the LV, less is known about their interrelationship in the RV subjected to chronic pressure load. TGF-β regulates growth, differentiation, and function of different cell lineages (eg, myocytes, endothelial cells, and fibroblasts) and has recently been implicated in maladaptive RV failure associated with pulmonary hypertension.[18] Furthermore, RV myocardial fibrosis, TGF-β signaling, and degradation of the matrix by MMPs have shown to be linked in earlier studies of chronic pressure overload and to adversely affect myocardial systolic and diastolic function.[19,20] In the LV, TGF-β signaling regulates *EDN1* expression via the JNK pathway, the effect of which in turn is regulated downstream by *CTGF*. Interestingly, the pattern of change in *CTGF* expression in our groups (a rise in the PAB group, which was attenuated in the PAB+AO group) was similar to those of TGF-β and *MMP9*, although the magnitude of difference did not reach statistical significance. We did, however, observe significantly increased myocardial expression of *EDN1* in both the PAB and the PAB+AO groups. Previous clinical studies of pulmonary hypertension have suggested that high circulating ET-1 levels are caused by increased gene expression and consequent synthesis, without a loss of the ET-1 clearance activity that normally occurs.[21-23] Whether our findings would be reflected by increased circulating levels of ET-1 or are limited to myocardial expression remains to be demonstrated, but it appears that the profibrotic responses to increased *EDN1* expression (which was similarly elevated in both PAB and PAB+AO groups, despite demonstrable differences in fibrosis by histologic examination) were in some way attenuated, presumably through regulation of upstream effects of reduced TGF-β or downstream through reduced receptor stimulation. With regard to the latter, our findings of decreased *EDNRB* expression may be important.

It was beyond the scope of this proof-of-principle study to explore fully the potential mechanisms by which aortic banding modifies the functional or molecular responses in the RV subjected to chronically increased afterload. A limitation of the study is that we are unable to comment on the mechanisms of adaptation with regard to the position of the ventricular septum. We speculate, however, that improved functional responses of the RV via the beneficial ventricular-ventricular interaction from increased LV afterload and increased contractility beneficially modifies the hemodynamic and myocardial milieu to reduce heart failure responses. Our finding of reduced MMP-9 signaling would be consistent with attenuation of myocardial oxidative stress,[24] itself a potent stimulator of adverse remodeling. In turn, adverse proinflammatory and profibrotic responses are attenuated, leading to more physiologic hypertrophy of the myocardium.

The potential therapeutic implications of our findings must also remain similarly speculative. Systemic vasoconstrictor therapy to recapitulate the effects of aortic constriction in patients would be neither easy to perform nor readily adopted by clinicians and patients. Our observations regarding the profibrotic responses within the RV do raise the possibility of using other agents to modify RV responses. Endothelin blockade is already widely used for patients with advanced pulmonary hypertension, but it might be argued that it would need to be given earlier in the course of the disease if it were to have a beneficial effect on RV remodeling. In this regard, modification of TGF-β signaling might be a more attractive target. It may be possible to modify fibrotic responses associated with increased TGF-β signaling through angiotensin II with an angiotensin receptor blocker, in the same way as has been shown in models of LV disease.[25]

In summary, we have shown that the functional and molecular maladaptive responses to increased RV afterload are attenuated by concomitant aortic constriction, presumably through beneficial ventricular-ventricular interactions. The molecular responses suggest modification of profibrotic pathways, which in themselves may be therapeutic targets to improve RV remodeling in chronic pulmonary hypertension and other conditions of RV pressure afterload.

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COMMENTARY

Aortic constriction and the relevance of physiologic research

Emile A. Bacha, MD

A casual reader might be tempted to skip over the article by Apitz and colleagues in this issue, dismissing it as irrelevant to clinical practice. The title is also somewhat off-putting because ''aortic constriction'' evokes only bad images in the mind of any cardiac surgeon. In fact, however, this elegant yet traditional cardiac physiology experiment shows that chronic aortic constriction not only improves right ventricular (RV) function in the setting of chronic RV pressure overload but also leads to less maladaptive RV remodeling. This article does inform on highly relevant themes, such as ventricle-ventricle interaction, timing of RV remodeling, and the molecular signature of RV profibrotic reactions. It may also open the door to a very untraditional way of managing the remodeled RV. The problem of the hypertrophied yet not decompensated RV is typically solved by an early repair of the anatomy, such as early repair of tetralogy of Fallot for example. No one would consider ''banding'' the aorta to allow for reverse RV remodeling. What, however, about the thick, fibrotic RV in an older patient with unrepaired pulmonary stenosis? Should we consider, akin to pulmonary artery banding and left ventricular training in an older patient with transposition of the great arteries, a period of aortic afterload increase to reposition the septum and maybe reverse some of the myocardial profibrotic signaling? What about managing the failing postoperative RV? In the setting of good left ventricular function, and in the absence of any current drugs that can directly increase RV contractility, systemic afterload increase by pharmacologic means may in fact have a role to play.

At a time when therapeutic answers are being sought mostly in genetics and tissue engineering, this study reminds us that physiologic research remains an essential and vital tool.

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CHD

Reversible pulmonary trunk banding. VI: Glucose-6-phosphate dehydrogenase activity in rapid ventricular hypertrophy in young goats

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Objective: Increased myocardial glucose-6-phosphate dehydrogenase (G6PD) activity occurs in heart failure. This study compared G6PD activity in 2 protocols of right ventricle (RV) systolic overload in young goats.

Methods: Twenty-seven goats were separated into 3 groups: sham (no overload), continuous (continuous systolic overload), and intermittent (four 12-hour periods of systolic overload paired with a 12-hour resting period). During a 96-hour protocol, systolic overload was adjusted to achieve a 0.7 RV/aortic pressure ratio. Echocardiographic and hemodynamic evaluations were performed before and after systolic overload every day postoperatively. After the study period, the animals were humanely killed for morphologic and G6PD tissue activity assessment.

Results: A 92.1% and 46.5% increase occurred in RV and septal mass, respectively, in the intermittent group compared with the sham group; continuous systolic overload resulted in a 37.2% increase in septal mass. A worsening RV myocardial performance index occurred in the continuous group at 72 hours and 96 hours, compared with the sham ($P < .039$) and intermittent groups at the end of the protocol ($P < .001$). Compared with the sham group, RV G6PD activity was elevated 130.1% in the continuous group ($P = .012$) and 39.8% in the intermittent group ($P = .764$).

Conclusions: Continuous systolic overload for ventricle retraining causes RV dysfunction and upregulation of myocardial G6PD activity, which can elevate levels of free radicals by NADPH oxidase, an important mechanism in the pathophysiology of heart failure. Intermittent systolic overload promotes a more efficient RV hypertrophy, with better preservation of myocardial performance and and less exposure to hypertrophic triggers. (J Thorac Cardiovasc Surg 2011;142:1108-13)

Supplemental material is available online.

Traditional pulmonary artery banding (PAB) aimed at ventricular retraining causes an abrupt and fixed systolic overload. Although clinical studies have proved that PAB induces myocardial hypertrophy, it is frequently preceded by ventricular dysfunction. Therefore, an adaptation period with inotropic support is generally required. Most important, previous studies have demonstrated myocardial edema and necrosis in hearts that experience abrupt systolic overload, followed by late ventricular failure.[1]

However, it is essential to understand the molecular mechanisms involved in PAB-induced myocardial hypertrophy to establish training protocols that lead to a desirable ''physiologic hypertrophy'' versus a deleterious ''pathologic hypertrophy.'' Because a known shift occurs in energy substrate use in favor of glucose in pathologic conditions, energy metabolism might be altered in PAB ventricular retraining protocols.[2] In addition, recent experimental studies have linked an unbalanced oxidative and reductive process to a variety of diseases, such as atherosclerosis and heart failure.[3]

Glucose 6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme that commits glucose to the pentose phosphate pathway, is mainly responsible for the generation of nicotinamide adenine dinucleotide phosphate (NADPH) and ribose 5-phosphate, an essential precursor of the de novo synthesis of RNA and DNA. G6PD-derived NADPH, a cofactor for glutathione and thioredoxin reductase, preserves reducing potentials and protects the cell from

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Abbreviations and Acronyms

G6P = glucose-6-phosphate
G6PD = glucose 6-phosphate dehydrogenase
LV = left ventricle
$NADP^+$ = oxidized nicotinamide adenine dinucleotide phosphate
NADPH = nicotinamide adenine dinucleotide phosphate
PA = pulmonary artery
PAB = pulmonary artery banding
RV = right ventricle

oxidative stress in normal conditions.[4] In human diseases, G6PD can be either activated or inhibited; however, evidence has emerged that the overexpression and activation of G6PD enhances NADPH oxidase–derived superoxide generation and increases oxidative stress in diseases like diabetes, heart failure, and hypertension.[5]

In regard to rapid ventricular training, it would be of great interest to study myocardial energy metabolism in response to different cardiac hypertrophy models and its relationship to heart function.[6] The main objective of this study was to compare the G6PD activity in 2 right ventricle (RV) training protocols through an adjustable PAB system.

METHODS

Twenty-seven young goats, aged between 30 and 60 days and of comparable weight ($P = .38$), were split into 3 groups: sham (n = 7; weight, 11.93 ± 2.67 kg), continuous (n = 9; weight, 10.74 ± 2.62 kg), and intermittent (n = 11; weight, 10.25 ± 2.20 kg). All animals received humane care in compliance with the guidelines established by the Brazilian regulations for animal experimentation. The protocol was reviewed and approved by the Ethics Committee for Research Protocols at the University of São Paulo Medical School.

Surgical Procedure

All operations were performed with the goats under general anesthesia (pentobarbital sodium 5 mg/kg intravenously and ketamine 20 mg/kg intramuscularly) and through a left lateral thoracotomy. The lung was retracted laterally to allow exposure of the right ventricular outflow tract, pulmonary trunk, and descending aorta. A 17-gauge heparinized catheter was inserted into each of these structures, and its corresponding pressures were measured at specific time intervals during the entire study. The adjustable PAB system (SILIMED; Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda, Rio de Janeiro, Brazil) was implanted just beyond the pulmonary valve and sutured at the adventitia of the pulmonary trunk, as previously described (Figure E1).[7] Antibiotics (cefazolin 500 mg and gentamicin 40 mg) were administered daily during the study, as were digoxin (0.04 mg/kg) and subcutaneous heparin (5000 U).

Training Protocol

RV systolic overload was initiated 72 hours postoperatively. Baseline hemodynamic data (RV, pulmonary artery [PA], and aortic pressures) were collected in a conscious, immobilized animal with the adjustable banding system deflated. Blood pressure measurements were obtained through computer software (ACQknowledge 3.01; Biopac Systems, Inc, Goleta, Calif). Then, the banding system was adjusted to achieve an RV/aortic pressure ratio of 0.7, limited by a 10% drop in systolic blood pressure. Adjustments were made just once, every morning throughout the protocol, by percutaneous injection of saline solution with a 3-mL syringe, under sterile conditions. That rule was violated in case of the latter occurring or if there were agitation, dyspnea, arrhythmia, or a combination of these. The banding system was then deflated up to a tolerable point.

Continuous Group Protocol

The animals remained with continuous systolic overload for 96 hours, with daily assessment to keep the RV/aortic pressure ratio at 0.7. Hemodynamic data were collected once a day (mornings) during PAB readjustments.

Intermittent Group Protocol

The animals underwent 4 daytime periods of RV 12-hour systolic overload, alternating with a 12-hour nighttime resting period. Hemodynamic data were collected twice a day (every 12 hours) during PAB readjustments.

Sham Group Protocol

The PAB system was maintained deflated during the entire protocol. Hemodynamic data were collected daily (mornings).

Echocardiography

A single experienced observer conducted the echocardiographic examination with the animals under light sedation (ketamine 15 mg intramuscularly) approximately 120 hours before the beginning of the protocol and daily thereafter until the end of the protocol. Image acquisition was obtained through 7.5-MHz and 2.5-MHz multifrequency transducers (Acuson Cypress Echocardiology System, Siemens, Erlagen, Germany). The following echocardiographic parameters were studied: left ventricle (LV), RV, and septal wall thicknesses, RV end-diastolic volume, and myocardial performance index.

Morphology

Animals were humanely killed after 96 hours of the study protocol. Cardiac samples were drawn from the RV, LV, and ventricular septum just before cardiac arrest. These samples were immediately frozen at –80°C (Forma Scientific Inc, Marietta, Ohio) to be subsequently analyzed for G6PD activity. The pericardial fat, both atria, and semilunar valves were dissected from the heart; RV, LV, and ventricular septum were separated by the Fulton technique, individually weighed (METTLER AE-200; Mettler-Toledo AG, Greifensee, Switzerland), and indexed to each animal's body weight.[8]

Water content was obtained individually in each cardiac chamber by subtracting the collected sample weight at autopsy from the weight of the dehydrated chamber (70 hours at 60°C). Values were obtained as a percentage of weight change.

G6PD Activity

Tissue samples were homogenized in extraction buffer (proportion 1:5 weight/volume). The material was stored in ice and homogenized for 30 seconds using Polytron (PT 3100; Kinematica AG, Littau-Lucerne, Switzerland) at maximum speed and centrifuging (15 kg, 15 minutes, 4°C) to separate from cell remnants. Enzymatic activity was analyzed using the supernatant of the last centrifugation. Proteins were quantified with the protein assay kit BCA (PIERCE Biotechnology, Rockford, Ill). Results are expressed as nmol min^{-1} mg^{-1} of protein. The extraction buffer for G6PD contained Tris-HCl (50 mmol/L) and ethylenediaminetetraacetic acid (1 mmol/L), with a pH of 8. The assay buffer (270 μL/sample) was Tris-HCl (8.6 mmol/L), $MgCl_2$ (6.9 mmol/L), (oxidized nicotinamide

adenine dinucleotide phosphate ($NADP^+$; 0.4 mmol/L), and Triton X-100 0.05% (volume/volume), with a pH of 7.6. The reaction was initiated by adding 15 μL of glucose-6-phosphate (G6P; 1.2 mmol/L) to the enzymatic extract (15 μL of sample) for 10 minutes at 25°C. The absorbance was monitored at 340 nm, the extinction coefficient being 6.22 for that particular wavelength. The biochemical reaction is based on the glucose phosphorylation into G6P, and posterior formation of 6-phosphogluconate by the action of G6PD. The G6PD activity was indirectly determined by the total production of NADPH through the pentose phosphate pathway.[9] RV and ventricular septum values were indexed to each animal's LV value.

Statistical Analysis

Values are expressed as means and standard deviation. Comparison of variables was performed with 2-way analysis of variance, except for body weight, G6PD activity, wall masses, and water content, which were performed with 1-way analysis of variance. Both analyses were followed by the Bonferroni post hoc test. The level of significance used was 5%. Statistical analysis was performed using GraphPad Prism version 4 software (GraphPad Prism, La Jolla, Calif) and SigmaStat 3.11.0 for Windows (Systat Software, Inc, Chicago, Ill).

RESULTS

Hemodynamic Variables

Systolic RV-PA gradient increased in both study groups ($P < .001$) and in every time period ($P < .001$). There was no significant difference of banding gradients between the 2 study groups on each day of the protocol, being both submitted to the same magnitude of systolic overload stimulus (Table E1). Likewise, both study groups showed an increased RV-aorta systolic pressure ratio over time (Figure E2) compared with its respective baseline and with that in the sham group at the same time ($P < .05$). There were peak systolic gradients in the intermittent group, alternating with 12-hour rest periods when they were similar to those in the sham group (Figure 1, *A*). The small RV-PA gradient (3.83 ± 1.33 mm Hg to 5.50 ± 1.38 mm Hg) observed in the sham group is considered physiologic. To quantify the exposure to systolic overload, we determined the area under the RV-PA gradient curve (Figure 1, *B*). The continuous group was the group most exposed to systolic overload ($P < .05$).

Echocardiographic Variables

RV wall thickness was thinner than septal and LV thicknesses in all animals preoperatively (group factor, $P = .663$; heart wall factor, $P < .001$). There was a marked increase in RV wall thickness after 48 hours in the intermittent group and after 72 hours in the continuous group compared with the baseline values (Table 1). The intermittent group developed a thicker RV wall (5.85 mm ± 1.32 mm) than the continuous group did (4.54 mm ± 0.51 mm) at 96 hours ($P < .001$). At the end of the training protocol, RV wall thickness increased 103.8% in the intermittent group and only 38.4% in the continuous group. No significant differences occurred in septal and LV wall thicknesses among groups ($P = .491$) and across time ($P = .865$). The graph with the percentage of changes in RV wall thickness is available online (Figure E3).

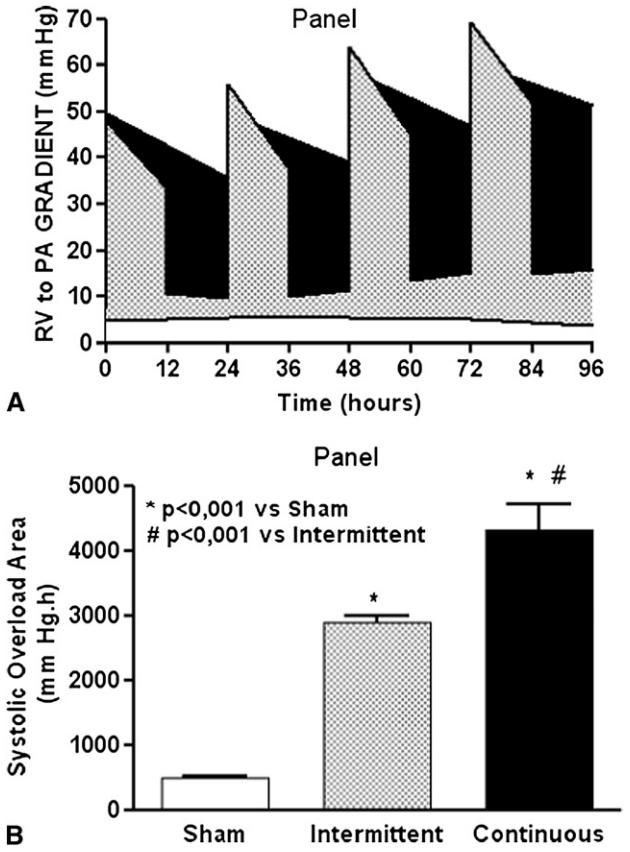

FIGURE 1. A, RV-PA gradient (mm Hg) temporal pattern of sham, continuous, and intermittent groups. B, According to variations in RV-PA gradients in relation to time, the area under the curve (mm Hg h) was calculated to determine the systolic overload imposed on each group. *RV,* Right ventricle; *PA,* pulmonary artery. *$P < .001$ compared with that of the sham group. #$P < .001$ compared with the intermittent group.

Regarding RV end-diastolic volume, there was a significant difference among groups ($P < .001$) and throughout the protocol ($P = .024$). RV volume was significantly dilated in the continuous group at 24 hours and thereafter, compared with that in the intermittent and sham groups

TABLE 1. RV wall thickness of the 3 groups measured by echocardiography in each time period of the protocol

	RV wall thickness (mm)		
Time (h)	Sham (n = 7)	Continuous (n = 9)	Intermittent (n = 11)
Preop	3.24 ± 0.14	3.28 ± 0.35	2.87 ± 0.58
0	3.30 ± 0.15	3.31 ± 0.33	2.98 ± 0.51
24	3.36 ± 0.11	3.37 ± 0.33	3.02 ± 0.59
48	3.39 ± 0.09	3.63 ± 0.68	3.91 ± 0.81*
72	3.37 ± 0.14	4.40 ± 0.64*,†	4.85 ± 0.66*,†
96	3.36 ± 0.08	4.54 ± 0.51*,†	5.85 ± 1.32*,†,‡

Values (mm) = means ± standard deviation. *$P < .001$ compared with its respective baseline value. †$P < .001$ compared with that in the sham group at the same time. ‡$P < .001$ compared with that in the continuous group at the same time.

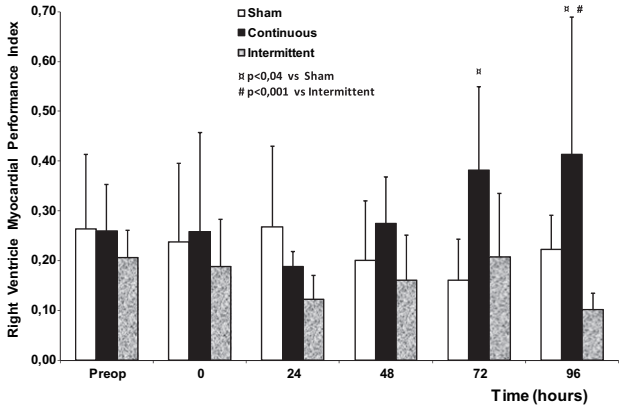

FIGURE 2. RV myocardial performance index during the 96-hour protocol in sham, continuous, and intermittent groups. *RV,* Right ventricle. ¤$P < .04$ compared with that in the sham group at the same time. #$P < .001$ compared with that in the intermittent group at the same time.

($P < .001$). The graph with the percentage of changes in RV end-diastolic volume is available online (Figure E4). Worsening of RV myocardial performance index was observed in the continuous group at 72 hours and 96 hours of the protocol, compared with that in the sham group ($P < .039$) and the intermittent group ($P < .001$) at the end of the protocol (Figure 2).

Morphologic Variables

As demonstrated in Figure 3, the intermittent group had a 92.1% increase in RV mass (1.46 ± 0.53 g/kg) and a 46.5% increase in ventricular septal mass (1.26 ± 0.29 g/kg) compared with the RV (0.76 ± 0.12 g/kg) and ventricular septal (0.86 ± 0.10 g/kg) masses in the sham group ($P < .05$). On the other hand, the continuous group had an increase only in the ventricular septal mass (1.18 ± 0.14 g/kg; $P < .05$). LV mass was not altered by RV training protocols ($P = .217$). Regarding water content, both RV (81.59% ± 1.07%) and ventricular septum (79.69% ± 0.62%) in the continuous group and both RV (81.84% ± 1.11%) and ventricular septum (79.45% ± 0.62%) in the intermittent group had a mild, however significant, increase compared with that in the sham group (RV, 78.84% ± 2.41%; ventricular septum, 77.11% ± 2.08%; $P < .01$). No significant changes were observed in LV water content in the 3 groups.

G6PD Activity

G6PD activity ratio of RV to LV was significantly elevated by 130.1% in the continuous group ($P = .012$), whereas the intermittent group showed a nonsignificant smaller increase of 39.8% in the G6PD activity ratio of RV to LV ($P = .764$) compared with that in the sham group (Figure 4). There was no significant difference in the ventricular septum to LV G6PD activity ratio among groups ($P = .198$).

DISCUSSION

The present experimental study aimed to compare PAB-induced RV hypertrophy in continuous versus intermittent systolic overload in young goats, with the emphasis on G6PD activity analysis. Both study groups were capable of promoting different degrees of myocardial hypertrophy compared with the sham group. However, the intermittent group had greater RV and ventricular septal masses than the continuous group had, despite less exposure to systolic overload. The mild increase in RV and ventricular septum water content in both trained groups would not by itself justify the previous findings, suggesting that it was probably related to the enhanced protein synthesis and cell proliferation, as previously documented by Abduch and colleagues.[10] The continuous group had a series of deleterious effects at the end of the protocol. Persistent RV dilation was followed by impaired RV function and increased G6PD activity, G6PD being the rate-limiting enzyme in the oxidative branch of the pentose phosphate pathway. Because the pentose phosphate pathway is one of the major sources of NADPH in cardiac myocytes, this is an important finding, and it may indicate an unbalanced redox, with the occurrence of oxidative stress and generation of reactive oxygen species related to NADPH oxidase.[11] In pathologic conditions, the excessive production of NADPH via G6PD overexpression is a result of multiple

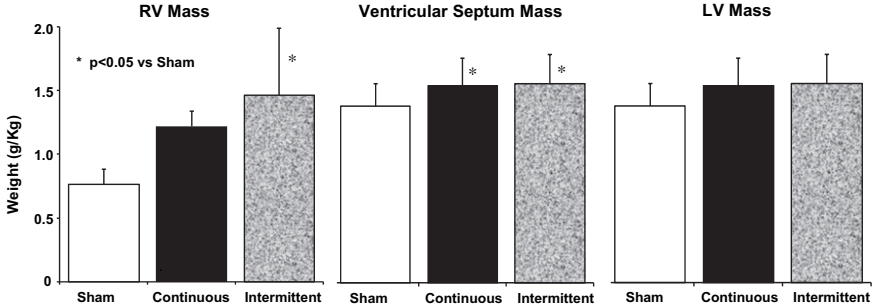

FIGURE 3. RV, ventricular septum, and LV weights in the 3 groups, indexed to body weight. Values (g/kg) = mean ± standard deviation. *RV,* Right ventricle; *LV,* left ventricle. *$P < .05$ compared with that in the sham group.

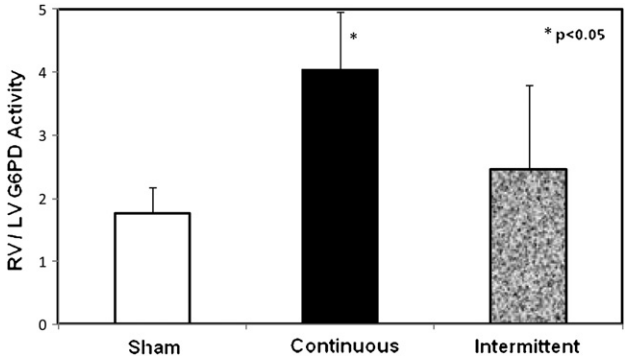

FIGURE 4. RV/LV ratio of G6PD activity in the sham, continuous, and intermittent groups. Values = mean ± standard deviation. Sham: n = 5; continuous: n = 5; intermittent: n = 10. *RV,* Right ventricle; *LV,* left ventricle; *G6PD,* glucose 6-phosphate dehydrogenase. *$P < .05$ compared with that in the sham and intermittent groups.

factors, such as angiotensin II, thrombin, and tumor necrosis factor alpha. The final consequence of this cascade of events would be the cardiomyopathy related to protein aggregation owing to reductive stress.[12,13]

It has been demonstrated that either G6PD activation or inhibition is associated with diseases. However, growing evidence has emerged that G6PD overexpression correlates with oxidative and reductive stress, and new investigational drugs are currently under development to suppress its action.[14] For instance, diabetic patients had upregulation of G6PD with high NADPH levels, and that was linked to inhibition of nitric oxide synthesis and endothelial dysfunction.[15] Although the mechanisms underlying the increased production of reactive oxygen species in the heart are not completely understood, it has been proposed that the high rate of glucose oxidation increases mitochondrial membrane potential, which enhances production of superoxide anion.[16,17] The latter would be a modulator in diabetic vasculopathy and precede the development of diabetic cardiomyopathy.[18,19]

Furthermore, in pacing-induced heart failure, an established model of dilated cardiomyopathy in dogs, it has been demonstrated that a 10-fold overexpression of G6PD occurs compared with that in controls.[20] Recent studies demonstrated that G6PD upregulation in mice adipocytes has been implicated as one of the causes of altered hormonal release observed in obesity and insulin resistance.[21]

Although the present work has not evaluated the generation of reactive oxygen species related to NADPH oxidase, it is tempting to speculate that, in case of persistent systolic overload, upregulation and hyperactivity of myocardial G6PD observed in the continuous group strongly suggest that the pentose phosphate pathway enhances cytosolic NADPH availability, thus fueling free radical production by NADPH oxidase and uncoupled nitric oxide synthase. Therefore, it may induce superoxide anion myocardial injury, as well as protein aggregation, and subsequently heart failure. However, other unknown mechanisms of G6PD in heart failure could not be ruled out. This issue has been investigated worldwide.

Regarding the training protocol, previous studies have demonstrated that ventricular training protocols induce myocardial hypertrophy 96 hours after progressive systolic overload.[22] The search for a physiologic hypertrophy has been the focus of our laboratory for over 2 decades, and we postulate that intermittent systolic overload promotes the desirable effects of myocardial hypertrophy without its adverse effects.[7] RV-PA gradients were more pronounced in the intermittent group after 48 hours of training. We would argue that the 12-hour resting period allowed the myocardium to replenish energy substrates and reestablish subendocardial perfusion owing to a lower ventricular wall tension. That would probably provide better hemodynamic performance at periods of systolic overload.[23]

Study Limitations

First, inferences based on animal findings do not necessarily translate into the same conclusions in humans. Second, RV hypertrophy was studied here, as opposed to human hearts with transposition of the great arteries. However, experimental models of aortic banding are associated with prohibited mortality rates.[24] It is difficult to make definitive conclusions about a hypertrophic process based on a single enzyme activity. Nevertheless, it is essential to correlate these biochemical findings with production of superoxide anions and apoptosis to better understand the role of oxidative stress in hypertrophy training protocols. Previous studies have demonstrated that the oxidative branch of the pentose phosphate pathway, which produces NADPH and ribulose 5-phosphate, is essentially irreversible, being controlled primarily by G6PD activity and, hence, the NADPH/NADP ratio.[25] NADPH oxidase preferentially uses NADPH derived from the pentose phosphate pathway, and that, in the failing heart, more glucose is oxidized through the pentose phosphate pathway, with a consequent increase in electron donors available to fuel O_2^- generating enzymes. Maybe that is the way $NADP^+$ is upregulated. Although this is a nonspecific pathway of free radical production, we have found a concordance of impaired RV function of continuous group and increased G6PD. Nevertheless, it would be more objective if we had measured oxygen-derived free radicals or tissue injury markers related to their production. Therefore, it is somehow difficult to assume and interpret a whole metabolic pathway based on the activity of a single enzyme. Future studies are being planned in our laboratory for the detection of increased oxidized glutathione, oxygen-derived free radicals, and myocardial injury markers that further supports the presence of a state of oxidative stress in the RV of the continuous group.

CONCLUSIONS

This study demonstrates that continuous systolic overload for ventricle retraining causes hyperactivity in myocardial G6PD, together with RV dilation and dysfunction. That enzyme hyperactivity may be related to an unbalanced redox determined by a constant and pathologic systolic overload. Given that pentose phosphate pathway enhances cytosolic NADPH availability, this altered energy substrate metabolism can elevate levels of free radicals by NADPH oxidase, an important mechanism in the pathophysiology of heart failure. On the other hand, intermittent systolic overload has promoted a more efficient RV hypertrophy than the continuous one, with better preservation of myocardial performance and smaller G6PD activity. Clinical studies comparing adjustable and traditional pulmonary trunk banding should be welcomed to translate those findings to the practice of ventricular retraining.

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Discussion

Dr William J. Brawn *(Birmingham, United Kingdom).* I have no disclosures. I have 2 questions.

In the human situation, acutely raising the ventricular ratio to 70% is usually followed by a fall in this ratio, probably with an associated falling cardiac output. How do you adjust the band, and how frequently, to maintain that 70% ratio in your animals?

Dr Atik. That is a very good point. Thank you for your question.

In our study, we performed the operation, and we have allowed the heart to recover for 72 hours before we initiated training. By the time we did it, we tightened the band to achieve that ratio. Sometimes we have to loosen it a little bit because there was a drop of more than 10% in systolic blood pressure or there were arrhythmias or agitation and hypoxia. So we went back to 0.5 or 0.6 ratio and waited a few minutes and then we tried it again. If it was not possible, we left the band tightened up to a tolerable point for the animal. Reassessments were made every 24 hours and in almost all the animals we had to readjust at that time.

Dr Brawn. Thank you. Final question, have you any insight as to how long it takes to upregulate the G6PD activity, how acutely it occurs?

Dr Atik. We do not have an answer for that question. This is a very good point. Actually, G6PD activity has a 2-fold mechanism. First, it enhances ribose 6-phosphate, which is a precursor of de novo production of DNA, which is good for hypertrophy. On the other hand, G6PD may enhance superoxide anion generation, which is bad for hypertrophy and can induce myocardial injury. So we really do not know how long does it take to modulate its activity.

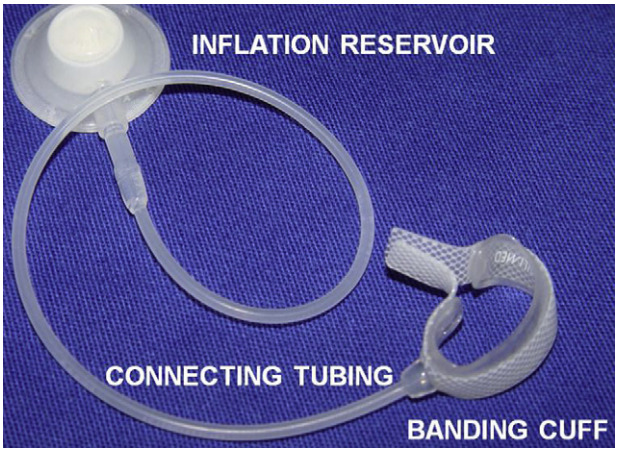

FIGURE E1. Pulmonary trunk adjustable banding device used in the protocol, manufactured by SILIMED, Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda, Rio de Janeiro, Brazil.

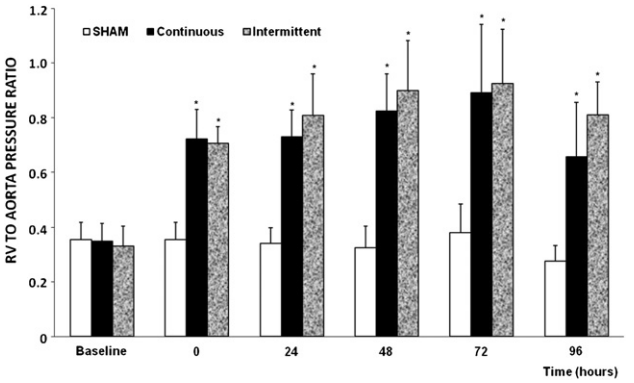

FIGURE E2. RV/aorta systolic pressure ratio of sham, continuous, and intermittent groups, throughout the protocol. *RV,* Right ventricle. *$P < .05$ compared with its respective baseline and with that in the sham group at the same time.

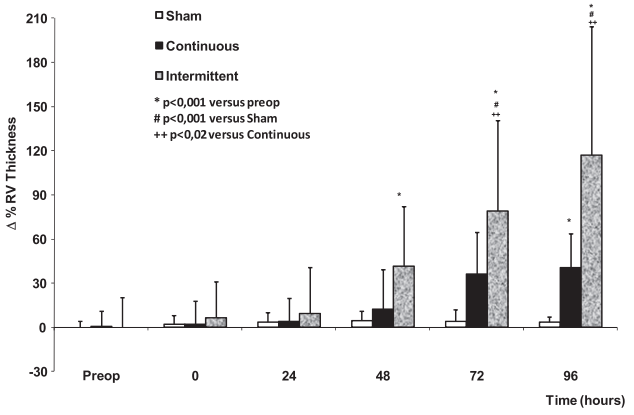

FIGURE E3. Δ% changes of RV wall thickness of sham, continuous, and intermittent groups throughout the protocol. *RV,* Right ventricle. *$P < .001$ compared with the respective baseline value. #$P < .001$ compared with the sham group at the same time. ++$P < .02$ compared with the continuous group at the same time.

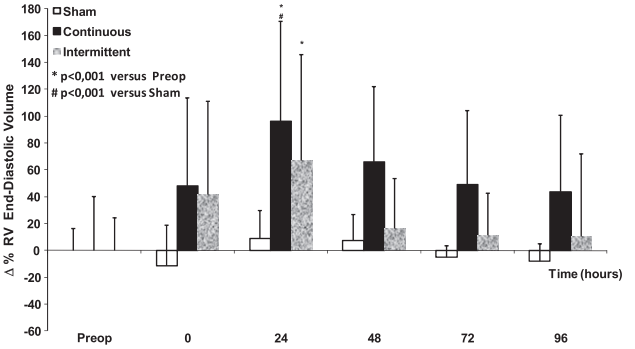

FIGURE E4. Δ% changes of RV end-diastolic volume of sham, continuous, and intermittent groups throughout the protocol. *RV,* Right ventricle. *$P < .001$ compared with the respective baseline value. #$P < .001$ compared with the sham group at the same time.

TABLE E1. RV-PA systolic pressure gradients of the 3 groups in each time period of the protocol

	RV-PA gradient (mm Hg)			
Time (h)	Sham	Continuous	Intermittent	*P* value*
Baseline	5.43 ± 1.99	8.00 ± 3.79	7.73 ± 6.40	1.00
0	5.43 ± 1.99	49.63 ± 16.70	48.00 ± 10.93	1.00
24	5.43 ± 1.27	49.43 ± 14.37	55.73 ± 9.91	.69
48	5.43 ± 1.13	58.88 ± 11.43	63.82 ± 13.69	.97
72	5.50 ± 1.38	60.88 ± 15.72	69.18 ± 9.78	.30
96	3.83 ± 1.33	51.29 ± 21.47	66.20 ± 11.37	.06

Values = mean ± standard deviation. *RV,* Right ventricle; *PA,* pulmonary artery. *P value of comparisons for continuous × intermittent groups.

Reversible pulmonary trunk banding III: Assessment of myocardial adaptive mechanisms—contribution of cell proliferation

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Objectives: Rapid ventricular conditioning induced by pulmonary artery banding has been recommended for patients with transposition of the great arteries who have lost the chance for the arterial switch operation or whose systemic (right) ventricle failed after the atrial switch. The present study was designed to experimentally evaluate 2 types of pulmonary artery banding (continuous and intermittent) and verify histologically the changes (hypertrophy or hyperplasia or both) of cardiomyocytes and vascular and interstitial cells from the stimulated ventricle beyond the neonatal period.

Methods: Twenty-one goats, 30 to 60 days old, were divided into 3 groups, each comprising 7 animals, as follows: control group (no surgical procedure); continuously stimulated group (systolic overload maintained for 96 hours); and intermittently stimulated group (4 periods of 12-hour systolic overload, alternated with a resting period of 12 hours). The animals were then killed for histologic and immunohistochemical analysis of the hearts. Murine monoclonal antibody Ki-67 was used as a proliferation cell marker. Myocardial collagen area fraction was determined by Sirius red staining.

Results: For both stimulated groups, a significant increase occurred in right ventricular cardiomyocytes and respective nuclei diameters compared with the controls ($P < .05$). The number of Ki-67-positive cardiomyocytes and interstitial/vessel cells from the right ventricle was augmented in both trained groups in relation to the left ventricle ($P < .05$). There was no significant difference in the right ventricular collagen area fraction from both trained groups compared with controls.

Conclusions: Irrespective of the shorter training time (periods of overload intercalated with resting), the intermittent stimulation regimen was able to produce a similar training of the subpulmonary ventricle compared with the continuous stimulation regarding mass acquisition, cell hypertrophy, and hyperplasia.

CHD

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Left ventricular retraining induced by pulmonary artery banding (PAB) has been indicated for patients with transposition of the great arteries (TGA) beyond the neonatal period and patients who present with right (systemic) ventricular failure in congenitally corrected TGA or after failed atrial baffle operations.[1,2] Previous experimental studies have demonstrated that an increase in afterload leads to sustained ventricular hypertrophy in only a few days.[3]

In the clinical setting of TGA, a few centers have documented the clinical results of rapid preparation of the left ventricle (LV) in the two-stage Jatene operation. These trials succeeded in their objective, once there was significant cardiac mass acquisition in about 7 days, reducing the risks of complications resulting from the first stage of the anatomical correction in a patient with unprepared LV. However,

Abbreviations and Acronyms
ANOVA = analysis of variance
LV = left ventricle
PAB = pulmonary artery banding
PT = pulmonary trunk
RV = right ventricle
TGA = transposition of the great arteries
VS = ventricular septum

some patients may have unsatisfactory contractile performance in the late follow-up.[2,4] Previous studies from our laboratory have demonstrated experimentally expressive muscular mass acquisition induced by an adjustable PAB system.[5,6]

At present, there is great concern about the quality of ventricular hypertrophy, leading to questions regarding the most efficient and physiologic training program and the adaptive mechanisms involved in the process.[1,2,7,8] On the other hand, the hypertrophy of an athlete's heart, characterized by normal or increased capillary density with little or no fibrosis, is a consequence of physiologic stresses like endurance exercise, intermittent by nature. Based on the fact that both the cardiac and the skeletal muscles are striated, it has been proposed that a fitness program similar to that developed by athletes would lead to an acquired muscular mass with better performance.[9,10] Besides that, the knowledge that the myocardium is a postmitotic organ, which means that cardiomyocytes are capable of proliferating after the neonatal period, leads to the hypothesis that myocyte hyperplasia may be an important feature in mass acquisition, although it has not been clarified to what extent it occurs.[11,12]

The present study was designed to experimentally evaluate 2 types of PAB (continuous and intermittent) and to analyze histologically the structural phenotype changes (hypertrophy and/or hyperplasia) of the contractile (cardiomyocytes) and noncontractile cells (vascular and interstitial) from the stimulated ventricle, beyond the neonatal period.

Materials and Methods

Twenty-one goats, 30 to 60 days old (beyond the neonatal period), were divided into 3 groups, each with 7 animals: control group, with no surgical procedure; continuously stimulated group; and intermittently stimulated group. All animals received humane care according to the "Guide for Care and Use of Laboratory Animals."[13]

Pulmonary Artery Banding Device

The device used in this study (Braile Biomedica, São José do Rio Preto, SP, Brazil) has been described previously.[5] It permits a fine control of the pulmonary blood flow percutaneously by adjusting accurately the cross-sectional diameter of the pulmonary trunk (PT). The prototype is made completely of silicone and consists of 3 parts: a banding ring, extension tube, and an inflation reservoir. The banding ring is a C-shaped hydraulic cuff that compresses the lumen of the PT when expanded, according to the volume injected percutaneously into the inflation reservoir, implanted subcutaneously.

Pulmonary Artery Banding Device Protocol

The anesthetic and surgical protocols were performed according to previously described techniques.[5] Catheters were introduced into the descending aorta, right ventricular outflow tract, and PT (distally to the banding) for hemodynamic measurements. Baseline pressures in the right ventricle (RV), PT, and aorta were then taken in the awake animals with the device completely empty. After that, progressive inflation of the device was achieved by percutaneous injection of saline solution in the reservoir to reach a 0.7 RV-to-LV systolic pressure ratio,[1,2,5] as long as it did not cause a drop of more than 10% in systemic pressure. In cases where clinical signs of severe hypoxia (agitation, dyspnea, or arrythmias) developed after inflation of the banding device, it was deflated to a tolerable value. The animals in the continuously stimulated group remained in RV systolic overload for 96 hours, with progressive inflations every 24 hours, at the maximum limit tolerated, while the intermittently stimulated group was submitted to 4 periods of 12-hour systolic overload, alternated with a resting period of 12 hours. Inflation of the device and pressure measurements were taken daily in both stimulated groups.

Echocardiographic Study

The echocardiographic evaluation was performed daily, using a 7.5-MHz transducer (Apogee CX, ATL—Advanced Technologies Laboratories, Bothell, Wash). The RV free wall mass was calculated through a bidimensional approach, according to the method described by Pontes and colleagues.[14] RV and LV ejection fractions were estimated by area × length and Teichholz methods, respectively.[15]

Morphologic Study of the Hearts

After the 96-hour protocol, the animals in the continuously stimulated and intermittently stimulated groups were killed.[5,13] All the hearts, including those from animals in the control group, were fixed in 10% buffered formalin for 24 hours. Transversal sections of both ventricles and the ventricular septum were obtained. After routine histologic processing, 5-μm sections were stained with hematoxylin-eosin and Sirius red. Sections also underwent immunohistochemical reactions with Ki-67.

Myocardial Fiber and Nucleus Diameter

The morphometric measurements were carried out with an interactive computer-assisted image analyzer (Leica Quantimet; Leica Cambridge Ltd, Cambridge, UK) in 60 longitudinally and/or transversely sectioned cardiomyocytes, as already described.[3]

Collagen Area Fraction

Histologic samples stained with Sirius red were studied for collagen area fraction by means of the computed image analysis system color detection (Quantimet–Leica). Data were collected from 20

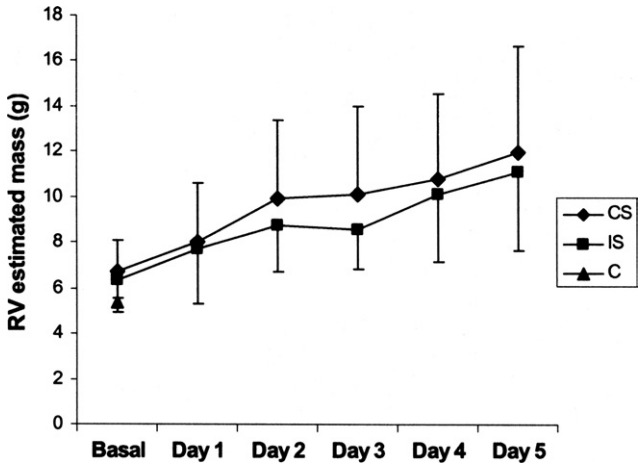

Figure 1. RV estimated mass (g) during training days. *RV*, Right ventricle; *C*, control group; *CS*, continuous stimulated group; *IS*, intermittent stimulated group.

fields at a magnification of 200×. Regions occupied by vessels greater than 50 μm or histologic artifacts were avoided.

Immunohistochemical Study

Five micrometer–thick sections underwent immunohistochemical reactions using the streptavidin-peroxidase immunohistochemical method. Murine monoclonal antibody Ki-67, clone MIB-1, code number IM0505 (DAKO, Grostrup, Denmark) was used as proliferation cell marker. According to previous studies, this marker has been used in goats.[16] Ki-67-positive cardiomyocytes and interstitial/vessel cells were microscopically quantified in the RV, LV, and ventricular septum (VS). The index of cell proliferation was calculated as follows: for each section of the heart (RV, LV, and VS), 4000 cells were counted (2000 cardiomyocytes and 2000 interstitial/vessel cells); the number of positive Ki-67 cells/2000 expressed as a percentage represented the index for each cell type. For the sections of the ventricular septum, the index was determined summing up the first 1000 cells counted in each half. Ultimately, for each group, the proportion between contractile and noncontractile elements labeled by the cell proliferation marker was determined.

Statistical Analysis

Values are expressed as means ± SE. Analysis of variance (ANOVA) was used to compare the 3 groups; for comparisons between the segments inside each group, the repeated measures ANOVA was used. When data did not show normal distribution, nonparametric tests were used.

Results

Hemodynamic Study

The intermittently stimulated group began the experiment with a 9.57 mm Hg basal RV-PT mean peak gradient (SE = 3.58 mm Hg). The last period inflation (72 hours) caused an 80.00 mm Hg gradient (SE = 4.92 mm Hg), which represents an increase equal to 736% ($P = .018$, Wilcoxon nonparametric test). In the continuously stimulated group, the basal mean peak gradient was 15.67 mm Hg (SE = 4.50 mm Hg). After 72 hours, this value was 57.14 mm Hg (SE = 5.22 mm Hg), which means a 265% rise in RV-PT pressure gradient ($P = .028$, Wilcoxon nonparametric test). Comparison between the 2 groups regarding the tolerated mean peak gradient indexed to the body weight revealed a significant difference, with greater values for the intermittently stimulated group after 72 hours of training ($P = .008$).

Right Ventricular Mass Estimated by Echocardiography

See Figures 1 and 2. Basal RV estimated mass was equal in the 3 groups (ANOVA, $P = .110$); however, in both trained groups, the right ventricular mass increased progressively throughout the protocol (ANOVA, $P < .05$). Right and left ventricular ejection fractions did not show significant changes in both stimulated groups during the study (mean values ± SE for the RV: controls, 0.71 ± 0.02; continuous stimulation, 0.67 ± 0.03; intermittent stimulation, 0.74 ± 0.03; mean values ± SE for the LV: controls, 0.70 ± 0.02; continuous stimulation, 0.75 ± 0.01; intermittent stimulation, 0.77 ± 0.01).

Collagen Area Fraction of the Right and Left Ventricles

Microscopically, no evidence of interstitial edema, relevant inflammatory infiltrate, or necrosis in the myocardium was

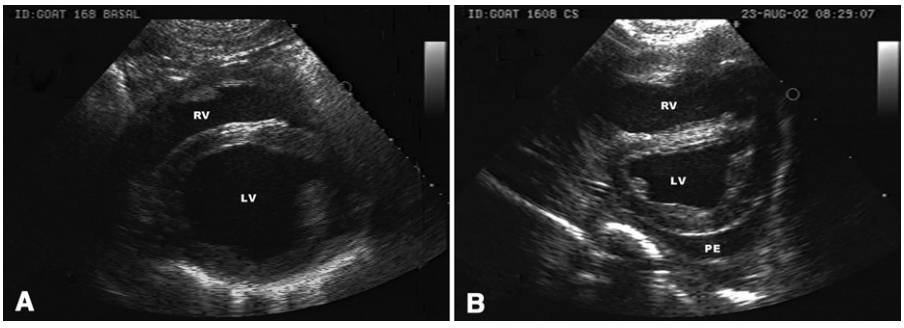

Figure 2. Bidimensional echocardiogram at baseline (A) and after 5 days of training (B) in a CS group goat. *RV*, right ventricle; *LV*, left ventricle; *PE*, pericardial effusion.

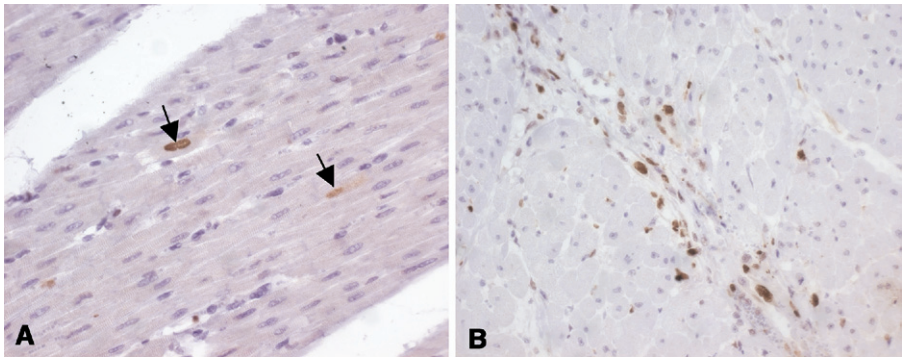

Figure 3. Photomicrographs of the RV myocardium showing Ki-67 immunostaining. A, *Arrows* indicate 2 labeled cardiomyocyte nuclei. B, Numerous positive nuclei from interstitial and vessel cells. Objective: 40×; counterstaining with Harry's hematoxylin. *RV*, Right ventricle.

present in the 3 groups analyzed. The mean collagen area fraction was significantly higher in the RV of the 3 groups when compared with the respective LVs (ANOVA, $P = .015$). There was no statistical difference in the RV collagen content between the groups (ANOVA, $P = .403$).

Cell Diameters

With regard to RVs, stimulated groups had significantly higher values of cell diameter than those of the control group, for both cardiomyocytes and their respective nuclei (ANOVA, $P < .005$). Compared with controls, myocyte diameters were 42.17% and 45.19% higher in the continuously stimulated and intermittently stimulated groups, respectively. Similarly, considering the cardiac segments inside the groups, RV cardiomyocytes and respective nuclei from the 2 stimulated groups had higher diameters than those of LV and ventricular septum (repeated measures ANOVA, $P < .05$).

Ki-67-positive Cardiomyocytes and Interstitial/Vessel Cells

The histologic aspect of Ki-67-labeled cardiomyocytes is demonstrated in Figure 3, *A*. Higher indexes of proliferating cells were found in the RV of both stimulated groups (continuously stimulated group, 1.13%; intermittently stimulated group, 0.68%) when compared with the corresponding LV (continuously stimulated group, 0.37%; intermittently stimulated group, 0.21%; repeated measures ANOVA, $P = .009$) and ventricular septum (continuously stimulated group, 0.21%; intermittently stimulated group, 0.08%; $P < .001$; Figure 4, *A*). The histologic aspect of the interstitial and vessel cells labeled with Ki-67 is demonstrated in Figure 3, *B*. In this analysis, the values obtained for the 2 halves of the ventricular septum were significantly different and were treated independently ($P = .024$). The right septal half had higher values compared with the left one. Analyzing the cardiac segments in the stimulated groups (Figure 4, *B*), RV had significantly higher indexes of cell proliferation than the LV (continuously stimulated group, 16.32% vs 2.57%; intermittently stimulated group, 12.36% vs 1.45%; repeated measures ANOVA, $P < .001$). The right septal half had statistically higher index values than did LV (continuously stimulated group, 3.73%; intermittently stimulated group, 4.94%; $P = .024$) and the left septal half (continuously stimulated group, 2.06%; intermittently stimulated group, 1.61%; $P = .012$). On the other hand, LV did not differ from the left septal half ($P = .527$). No difference was detected in the cardiac segments of the control group (repeated measures ANOVA, $P = .198$).

Proportion Between Numbers of Ki-67-labeled RV Cells (Cardiomyocytes/Interstitial and Vessel Cells)

Statistical analysis of these data did not depict any difference between the 3 groups (Kruskal–Wallis, $P = .4320$).

Discussion

Induced myocardial hypertrophy has been proposed in children with TGA, aiming at preparing the future systemic ventricle to support the new hemodynamic conditions after the anatomical correction (Jatene's procedure).[2,17] The adaptation of the pulmonary ventricle to the systemic pressures must be effective, which means that the chamber has to keep the mass acquisition and not experience dilation or dysfunction over time. Based on the fitness program of athletes, where the skeletal and cardiac muscles are capable of sustaining a high workload through fiber hypertrophy, a hypothesis arose that perhaps intermittent conditioning of the subpulmonary ventricle could provide better adaptation.[9,10] However, no consensus exists about what would be the ideal time to impose systolic workload on a ventricle, inducing it to acquire good-quality muscular mass.

Pressure overload induces an increase in cardiac wall thickness without chamber dilation (concentric hypertrophy) for the purpose of normalizing systolic wall stress.[18] It is fundamental to determine the primary mechanism of mass acquisition (ie, cell hypertrophy, hyperplasia, or both). Hypertrophy is efficient as an adaptive mechanism in the compensated phase but may evolve into muscle dysfunction and congestive heart failure with time.[19] Nowadays, it is fully accepted that cardiomyocytes are capable of prolifer-

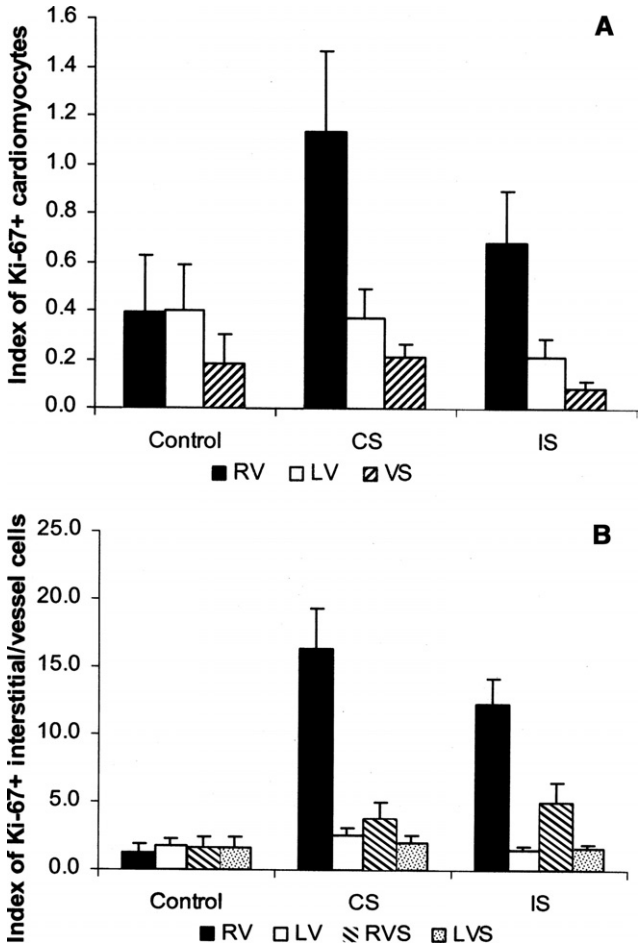

Figure 4. Mean and SE values of: (A) cardiomyocytes Ki-67-positive index (number of positive cells/2000 cardiomyocytes expressed as a percentage); (B) interstitial/vessel cells Ki-67-positive index (number of positive cells/2000 expressed as a percentage). *RV*, right ventricle; *LV*, left ventricle; *RVS*, right half of ventricular septum; *LVS*, left half of ventricular septum.

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ating after the neonatal period. During fetal life, cardiac growth is mainly due to cell division (mitosis). In the neonatal period, a transition occurs from hyperplastic to hypertrophic growth. In the adult heart, most cardiomyocytes do not proliferate, and hypertrophy functions as the fundamental adaptive response.[20] Therefore, the magnitude of cardiomyocyte hypertrophy or hyperplasia is dependent on the age at which the stimulus is produced.[4,8,12]

Myocardial Hypertrophy

In the present study, the echocardiogram showed a similar right ventricular mass acquisition for both stimulated groups. The RV free wall thickness, an important parameter in ventricular mass calculation, became similar to the septal and left ventricular wall thicknesses around the third day of device inflation in both groups, despite the fact that 1 of them was stimulated intermittently. Morphometric analysis revealed that for both stimulated groups, hypertrophy of cardiomyocytes occurred, confirming that this mechanism participates in the process of muscle mass acquisition during ventricular training, as has already been demonstrated in other pressure-overload experimental models.[18]

In myocardial hypertrophy caused by other factors, it is well recognized that interstitial cell proliferation is also a feature. The interstitium has important functions, such as support for cardiomyocytes, blood and lymphatic vessels; acting as a defense mechanism against microorganisms; facilitating myocardial nutrient exchanges; and aiding in cell contraction. However, when interstitium enlargement is excessive, it may cause early diastolic dysfunction and, in the final stages, also jeopardizes systolic function.[21] In this study, no significant difference existed in RV collagen area fraction between the 3 groups. We cannot rule out the possibility that the observation time was not sufficient to demonstrate an increase in the interstitial component. Le Bret and colleagues,[10] comparing 3 types of pulmonary ventricular training in lambs for 5 weeks, demonstrated no interstitial fibrosis in animals undergoing the intermittent regimen of training in opposition to the other groups, suggesting that the cascade of events initiated with myocardial hypertrophy is not accompanied by fibrosis when it develops in good oxygen conditions (corresponding to the period that the ventricle does not experience an afterload increase). Buccino et al,[22] studying 26 cats with right ventricular hypertrophy produced by continuous PT constriction for a period of 3 to 90 days, encountered higher collagen concentrations in RVs compared with that in the controls and LVs. The majority of RVs failed after 20 to 50 days. Therefore, it seems that the duration of the training period is important as an inductor of myocardial fibrosis responsible for late heart failure.

Cell Proliferation

The present study showed that both cardiomyocytes and interstitial/vessel cells are able to improve their capacity for proliferation, under continuous or intermittent elevation in afterload, even beyond the neonatal period. It is important to note that the intermittent regimen of PAB, which potentially could be better tolerated clinically, is as effective as the continuous regimen in inducing RV cardiomyocyte hyperplasia. Although the idea of having a high proliferation index for contractile myocardial cells would be tempting in this model, it must not be forgotten that an increase in their number will also augment the demand for oxygen and nutrients and, consequently, for blood vessels, to maintain the needs of proliferating cells.[9,18] So, it would be desirable to have a balance between myocardial cells and capillary vessel proliferation, promoting a ventricular conditioning

that is considered ideal to sustain the requirements of systemic circulation.[23] Anversa and associates[9] submitted 5-week-old Wistar–Kyoto rats to intermittent physical training by having them run on a treadmill 1 h/d, 5 d/wk, for 7 weeks. At the end of the experiment, they found a 16% increase in myocardial capillary vessels/mm^2, indicating that vascular cell proliferation did occur in this physiologic model of fitness.

It is not easy to distinguish between interstitial cells (mainly fibroblasts) and capillary endothelial cells under optical microscopy, because the small vessels are not always open. Therefore, in the present study, it is not possible to confirm whether the Ki-67-labeled interstitial/vessel cells are predominantly endothelial cells or fibroblasts. Double-labeling immunohistochemical reactions may elucidate this question in the future.

Regarding the proportion of Ki-67-positive cardiomyocytes and interstitial/vessel cells, no statistical difference existed between the groups (Kruskall–Wallis, $P = .432$). Hence, apparently, both fitness regimens are capable of inducing a proportionally equal increase in contractile and noncontractile elements after a period of 96 hours of training.

Myocyte proliferation has been demonstrated under normal and pathologic conditions, such as heart failure or acute myocardial infarction.[24,25] In the adult heart, a subpopulation of cardiomyocytes exists that is not differentiated, capable of reentering the cell cycle and proliferating. There is no definitive evidence about the exact origin of these undifferentiated cells. They may come from itinerant cells or even from myocardial stem cells. These hypotheses need further investigation.[24] Several researchers have also verified that the capacity of hyperplasia is accompanied by apoptosis.[24-28] Both clinical[24] and experimental[28] investigations showed that diseased hearts present, simultaneously, signs of cardiomyocyte hyperplasia and programmed cell death. The studies suggest that tissue homeostasis depends on proper relationships between proliferation, differentiation, and apoptosis, so that under severe stress, apoptosis overtakes cell division.[28] Concomitant cell proliferation and apoptosis would have important implications when a ventricle is prepared to support systemic pressures, once it is desirable that contractile cells proliferate and remain preserved. From a therapeutic standpoint, an index lower than 0.05% to 0.1% of proliferating cardiomyocytes would be sufficient to significantly increase the number of contractile cells in a few months, because this growth takes place in a sustained manner, which means without being overcome by apoptosis.[20] In the present study, training groups had indexes of 1.13% (continuously stimulated group) and 0.68% (intermittently stimulated group). However, we did not search for apoptosis in this model.

Another substantial factor to be analyzed in the future would be the quantification of capillaries in the 3 groups studied. The type of training that demonstrates the best balance between contractile cell proliferation and blood vessels, besides the smallest apoptotic index, would be considered as the most adequate to be used in the 2-stage arterial switch operation.

The finding of a higher proliferation index for cells in the right half of the ventricular septum deserves discussion. Feigenbaum[29] long ago described in echocardiography the presence of a bright line within the ventricular septum, dividing its 2 sides. Boettler and colleagues,[30] studying hearts from 30 healthy subjects, demonstrated, also through echocardiography, that this line probably represents the VS division in 2 halves (right and left). Results from the present study suggest a different behavior of the 2 septal halves when the RV undergoes pressure overload and corroborate the theories of septal division mentioned above.

Limitations of the Study

The main limitation of this study is the fact that differences exist between the RVs and LVs with respect to anatomy and physiology. One could argue that experimental results would not be the same if the trained ventricle was the morphologically left one. However, even having knowledge of this limitation, the option to work with healthy animals prevailed because the surgical procedure to change the ventriculoarterial connections would cause high mortality, making the research impracticable.[6] Moreover, although at first sight RV training could represent a limitation because of the different geometry of the chamber, considering that in our experimental model the coronary arterial circulation is not submitted to a hypertensive regimen due to pressure overload, one can observe that this situation is similar to the one that occurs in the child with transposition requiring ventricular preparation, which would be an advantage. Another restriction to consider is the training time in our 2 groups: animals prepared continuously had PAB for 96 hours, and in the intermittently stimulated group, animals were trained for 48 hours. However, in this study, the intention was to analyze the behavior of the ventricles that underwent rapid preparation, totaling a week of study. The final objective of this line of research is to obtain the best results in the shortest time possible, aiming at optimal clinical application.

Conclusions

Under the conditions of the present study, the intermittent stimulation regimen, even imposing a shorter training time (periods of overload intercalated to periods of resting), was able to induce a similar response of the myocardium regarding cell hypertrophy and hyperplasia when compared with the continuous stimulation. Moreover, although there was

an increase in the interstitial cellular activity, it did not impair right ventricular systolic function (represented by the ejection fraction) in both stimulated groups.

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Reversible pulmonary trunk banding. II. An experimental model for rapid pulmonary ventricular hypertrophy

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Objective: An experimental model with a reversible pulmonary trunk banding device was developed with the aim of inducing rapid ventricular hypertrophy. The device consists of an insufflatable cuff connected to a self-sealing button.

Methods: The right ventricles of 7 young goats (average weight, 8.7 kg) were submitted to systolic overload and evaluated according to the hemodynamic, echocardiographic, and morphologic aspects. Baseline biopsy specimens were taken from the myocardium for microscopic analysis. The device was implanted on the pulmonary trunk and inflated so that a 0.7 right ventricular/left ventricular pressure ratio was achieved. Echocardiographic and hemodynamic evaluations were performed every 24 hours. Systolic overload was maintained for 96 hours. The animals were then killed for morphologic study. Another 9 goats (average weight, 7.7 kg) were used for control right ventricular weight.

Results: The systolic right ventricular/pulmonary trunk pressure gradient varied from 10.1 ± 4.3 mm Hg (baseline) to 60.0 ± 11.0 mm Hg (final). Consequently, the right ventricular/left ventricular pressure ratio increased from 0.29 ± 0.06 to 1.04 ± 0.14. The protocol group showed a 74% increase in right ventricular mass when compared with the control group. Serial 2-dimensional echocardiography showed a 66% increase in right ventricular wall thickness. There was a 24% increase in the mean myocyte perimeter, and the myocyte area increased 61%.

Conclusions: The device is easily adjustable percutaneously, enabling right ventricular hypertrophy in 96 hours of gradual systolic overload. This study suggests that the adjustable pulmonary trunk banding might provide better results for the 2-stage Jatene operation and for the failed atrial switch operations to convert to the double-switch operation.

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The Jatene operation is the treatment of choice for transposition of the great arteries (TGA).[1,2] Retraining of the left ventricle is necessary in patients with TGA beyond the neonatal period and in congenitally corrected TGA or after Senning or Mustard operations with right ventricular (RV) failure. In these cases the repair should be performed in 2 stages.[3,4]

We still have a considerable number of patients with late referral for the Jatene operation. However, we were not able to reproduce the excellent Boston experience of rapid left ventricular (LV) preparation,[4] probably related to the lack of pulmonary

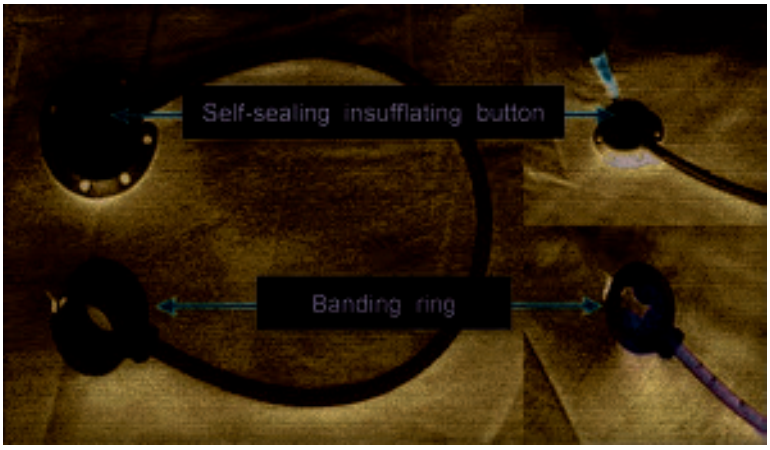

Figure 1. PT banding device consisting of 3 parts: banding ring, extension tube, and self-sealing inflation button.

trunk (PT) banding adjustment, which has been done empirically. The degree of PT banding might be inadequate or imprecise, causing an important acute systolic overload on the left ventricle, which in turn would impair late ventricular function.[5-8]

We developed an experimental model designed to adjust PT banding percutaneously and to induce rapid pulmonary ventricular hypertrophy. The RV changes caused by the PT banding device were evaluated according to hemodynamic, echocardiographic, and morphologic aspects after 96 hours of uninterrupted gradual systolic overload.

Materials and Methods

Anesthesia

Seven young goats, 30 to 60 days old and with a mean weight of 8.7 ± 2.4 kg, were studied. Another 9 goats, with similar age and a mean weight of 7.7 ± 1.2 kg, were used as a control group for RV weight evaluation. Anesthesia was induced with ketamine (50 $mg \cdot kg^{-1}$). A jugular venous line was placed for drug infusions. Each animal was then sedated with pentobarbital sodium (Nembutal; 5-10 $mg \cdot kg^{-1}$ administered intravenously) and then ventilated through a tracheal tube with 100% oxygen (Harvard 708). Electrocardiographic and blood pressure measurements were taken through computer software (ACQknowledge 3.01, Biopac Systems, Inc). The goat was then prepared for sterile surgical procedure. Goats received 500 mg of cefazolin and 10 mg of gentamicin intramuscularly every 12 hours, beginning just before the operation.

PT Banding Device

Figure 1 shows the 3 components of the banding device: banding ring, extension tube, and insufflation button. The banding ring (Hazen Everett Co) is a U-shaped hydraulic cuff with a 10-mm internal diameter and a 5-mm width. Its outer layer consists of 1-mm-thick rigid silicone, which keeps it from deforming. The inner surface has a deformable layer of silicone, which expands, compressing the lumen of the vessel according to the volume injected into the inflation button. At the 2 ends of the cuff, there are small orifices that are used for securing the ring to the PT. The extension tube, also made of silicone, links the banding ring with the insufflation button. It has a 2-mm inner diameter and is 25 cm long. The inflation button (Bard Access System) is a circular reservoir made of self-sealing silicone, the base of which includes a metal plate. The reservoir has a port, which is connected to the extension tube. This button is implanted subcutaneously, thus permitting the inflation or deflation of the banding ring percutaneously.

Procedure

The chest was opened at the fourth left intercostal space to expose the descending aorta and the RV outflow tract. A purse-string suture (5-0 polypropylene) was placed in the aorta to insert a 16-gauge catheter (Bard Co) for systemic blood pressure monitoring. Two additional 16-gauge catheters were implanted, one in the right ventricle and another distally in the PT, and fixed in position with a 5-0 polypropylene purse-string suture. All the catheters were kept heparinized and then exteriorized through the chest wall. The PT was then dissected free for banding device placement. The banding ring was wrapped around the PT, and its ends were sutured together and secured on the adventitia with polypropylene 5-0 sutures just above the pulmonary valve level. The extension tube of the banding ring was brought out through the third intercostal space and connected to the insufflation button, which was lodged subcutaneously in the chest wall. The inflation button was tested, and all of the air in the system was evacuated. The ribs were approximated after placing a small drainage catheter in the left pleural space, and the soft tissues were closed. After 4 to 6 hours of postoperative care, the pleural catheter was then removed.

Echocardiographic Studies

All examinations included 2-dimensional and M-mode echocardiographic imaging of the ventricles from the right parasternal view with 2.5- and 5-MHZ transducers (Ultramark 4, Advanced Technology Laboratories). Initial evaluation confirmed a RV free wall thickness smaller than that of the left ventricle. Postoperative

TABLE 1. Hemodynamic data before and after the 96-hour systolic overload of the right ventricle (n = 7)

RV systolic overload	*Heart rate (beats/min)*	*Systemic systolic pressure (mm Hg)*	*RV systolic pressure (mm Hg)*	*RV gradient (mm Hg)*	*RV/LV pressure ratio*
Baseline	127.9 ± 12.5	79.4 ± 6.1	22.4 ± 4.1	10.1 ± 4.3	0.29 ± 0.06
96 h	150.0 ± 8.7	68.6 ± 3.6	71.0 ± 10.0	60.0 ± 11.0	1.04 ± 0.14
P value	.01	.005	.0001	.0001	.0001

Values are presented as means ± SD.

evaluation of induced hypertrophy was performed with intervals of 24 hours. The following parameters were observed: (1) RV, LV, and septal wall thicknesses; (2) RV/PT pressure gradient; and (3) presence of effusions.

Protocol

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. Digoxin was given to all goats (0.5 $\mu g \cdot kg^{-1} \cdot d^{-1}$ administered intramuscularly). The inflation protocol of progressive RV systolic overload was begun after full surgical recovery. After taking baseline pressure measurements, the PT banding device was initially inflated by means of percutaneous injection of saline solution with a 1-mL syringe under sterile conditions to achieve a 0.7 RV/LV systolic pressure ratio, as long as it did not cause more than a 10% systemic pressure drop. The amount of water used for cuff inflation varied from 0.1 to 0.4 mL. The volume in the banding device was progressively incremented at 24-hour intervals, according to animal tolerance to the pressure load. If systemic hypotension, respiratory distress, or both developed after inflation of the banding device, it was deflated to the previous volume compatible with maintenance of goat hemodynamics. Hemodynamic evaluation was performed every 24 hours. The RV systolic overload was carried out for 96 hours. The animals were then killed for morphologic evaluation of the heart.

Morphologic Studies

Baseline myocardial samples (3 mm maximal diameter) from the subepicardial layer of the RV outflow tract were collected for optical microscopic studies just before catheter insertion. After 96 hours of RV systolic overload, the animals were killed, and the positions of the catheters were checked in the right ventricle, PT, and aorta. The hearts were then removed from the thorax. Similarly, the control group animals were also killed to measure the ventricular and septal weights.

Weight determinations. The ventricular and septal weights were taken according to the Fulton technique (METTLER AE-200, Mettler-Toledo AG).[9] The weight measurements were indexed to the body weight of the animal (in grams per kilogram), as suggested by Bishop and Cole.[10]

Optical microscopy. After weight measurements, the hearts were fixed in 10% buffered formalin for 24 hours. A transverse cut of the ventricular mass was made 1 cm below the level of the atrioventricular junction. Sections from the ventricular septum, right ventricle, and left ventricle were obtained. After routine histologic processing, 5-μm sections were stained with hematoxylin and eosin. The perimeter and area of transversely sectioned cardiomyocytes were measured at the level of the nucleus by means of an image analysis system (Quantimet-Leica, Leica Cambridge Ltd) at a magnification of 400×. Data were collected from 140 myocytes of baseline samples and from 105 myocytes of the ventricles subjected to 96 hours of RV systolic overload.

Statistical Analysis

Values are expressed as means ± SD. The paired Student *t* test was used to compare the baseline data with the 96-hour data of the RV systolic overload group under hemodynamic, echocardiographic, and optical microscopy aspects. The unpaired Student *t* test was used (GB-STAT, Dynamic Microsystems) to compare the septal and ventricular weights. The statistical significance was set at 5% level.

Results

During the protocol, the animals remained active, with no clinical signs of RV decompensation. All the implanted devices functioned adequately for water injection and suction. No migration or rupture of the device was noted nor was there any injury to the adjacent structures caused by the cuff or leaks through the single connection between the extension tube and the self-sealing insufflation button.

Hemodynamic Measurements

All the animals presented with tachycardia at the end of the protocol (baseline, 127.9 ± 12.5 beats/min; after 96 hours of systolic overload, 150.0 ± 8.7 beats/min; $P = .003$). We noticed a 13.6% decrease in the aortic systolic pressure during the protocol, although no changes in LV function were observed with echocardiography ($P = .005$).

After 96 hours of systolic overload, the right ventricle showed a 217.0% increase in systolic pressure, from 22.4 ± 4.1 mm Hg to 71.0 ± 10.0 mm Hg, surpassing the systolic pressure of the left ventricle ($P = .0001$). The RV/PT systolic gradient varied from 10.1 ± 4.3 mm Hg to 60.0 ± 11.0 mm Hg ($P = .0001$), whereas the RV/LV pressure ratio increased from 0.29 ± 0.06 (before RV systolic overload) to 1.04 ± 0.14 (after the 96-hour protocol, $P = .0001$). The hemodynamic data are summarized in Table 1.

Table 2 shows the time course of RV/LV pressure ratio response for each animal. At the initial banding, 3 animals (nos. 2, 3, and 5) did not achieve a desired level of banding.

TABLE 2. Time course of RV/LV pressure ratio response for each animal

	Period of protocol (h)								
	Baseline		24		48		72		96
Animal	Found	Left	Found	Left	Found	Left	Found	Left	Found
1	0.32	0.81	0.49	0.89	0.75	0.90	0.80	1.10	1.00
2	0.20	0.48	0.32	0.59	0.27	0.73	0.42	0.95	1.01
3	0.24	0.61	0.33	0.96	0.56	1.04	1.07	1.07	1.01
4	0.28	0.69	0.51	0.77	0.44	0.94	1.33	1.33	1.13
5	0.23	0.51	0.36	0.71	0.32	0.71	0.53	0.56	0.79
6	0.35	0.93	0.80	0.91	0.84	0.96	0.97	1.11	1.09
7	0.37	0.93	0.88	0.92	0.80	1.05	0.93	1.06	1.22

Values are RV/LV pressure ratios of each animal subjected to 96 hours of systolic overload of the RV. *Found,* Pressure ratio encountered during daily measurements before adjustments; *left,* daily pressure ratio adjusted.

The RV/LV pressure ratios were 0.48, 0.61, and 0.51, respectively. During the protocol, 2 animals did not tolerate more banding at 72 hours (animal 3, RV/LV ratio of 1.07; animal 4, RV/LV ratio of 1.33). Nevertheless, RV hypertrophy was achieved in all of the animals.

Echocardiographic Findings

Table 3 shows the echocardiographic data before and after RV overload. We observed a 66% increase in the RV free wall thickness (from 4.4 ± 0.5 mm to 7.3 ± 1.7 mm, $P = .002$), surpassing that of the LV free wall and the septum (5.1 ± 0.4 mm). There were no significant changes in the septum and LV free wall thicknesses. The RV/PT pressure gradient, measured by means of Doppler echocardiography, increased from 10.6 ± 5.4 mm Hg before the protocol to 74.9 ± 9.7 mm Hg after the 96-hour period of systolic overload ($P = .0001$). The catheter pressure gradient measurements are overestimated by 5% before the protocol and by 25% at the end of 96 hours of systolic overload. Pleural effusions were found in all the animals.

Morphologic Findings

The intimal surface of the PT appeared normal. None of the animals demonstrated macroscopic thickening of the pulmonary valve.

Weight of the ventricles. The RV weight from the studied animals was 74% greater than that from the control group (0.99 ± 0.17 vs 1.72 ± 0.24 g/kg, $P = .0001$). There was no difference between the septal and LV weights from the studied animals compared with those in the control group. These weight measurements are summarized in Table 4.

Optical microscopy. Table 5 shows the results of the perimeter and area of the cardiac fibers measured before and after the RV systolic overload. A 27% increase in the perimeter of the RV myocyte was found after 96 hours of systolic overload (from 45.9 ± 4.5 μm for the baseline condition to 58.3 ± 9.3 μm after the protocol, $P < .0001$). Figure 2 presents representative pictures of the RV cardiac myocytes of animal 6 before and after the protocol. We noted a 69% increase of the cardiac myocyte area after the protocol (from 132.2 ± 25.4 μm^2 for the baseline condition to 223.5 ± 72.1 μm^2 after the protocol, $P < .0001$). We did not observe significant signs of interstitial fibrosis or extracellular edema.

Discussion

The device presented in this study represents an alternative approach not only for the LV rapid preparation in patients with TGA and intact ventricular septum beyond the neonatal period but also in converting the failed Mustard or Senning procedure to the Jatene operation or in congenitally corrected TGA with RV failure.[11-13] It was designed for variable and reversible manipulation of the subpulmonary ventricular afterload, regardless of the time required to achieve that manipulation. Thus a progressive systolic overload can be applied to the subpulmonary ventricle, limiting the abruptness of the acute stenosis imposed during conventional PT banding.

Critique of the Preparation

This protocol analyzes some aspects of the acute RV hypertrophy in a young animal model. At the present time, it is not clear whether the response to the stimulus of the variable systolic overload on the subpulmonary ventricle of adult animals would be the same. In the clinical setting the hypertrophic response of the subpulmonary ventricle has been demonstrated to take a longer time. According to Mavroudis and Backer,[12] patients with TGA and failed atrial inversion aged from 1.9 to 23 years took an average of 15.6 months to retrain the subpulmonary ventricle for the 2-stage Jatene operation. Ongoing studies in our laboratory are assessing this protocol in an adult model. Blood oximetry changes induced by PT banding were not evaluated in this study. However, no clinical signs of significant hypoxia, acidosis, or both were observed. Although a more precise adjustment of RV systolic overload could be

TABLE 3. Echocardiographic findings before and after the 96-hour systolic overload of right ventricle (n = 7)

RV systolic overload	*RV free wall (mm)*	*Interventricular septum (mm)*	*LV free wall (mm)*	*RV/PT gradient (mm Hg)*
Baseline	4.4 ± 0.5	4.9 ± 0.4	5.4 ± 0.5	10.6 ± 5.4
96 h	7.3 ± 1.7	5.1 ± 0.4	5.1 ± 0.4	74.9 ± 9.7
P value	.002	NS	NS	.0001

Values are presented as means ± SD. *NS*, Not significant.

achieved, this model does not reflect the clinical reality of patients with TGA and a functionally intact ventricular septum. PT banding might not be tolerated in patients with significant hypoxia without a Blalock-Taussig shunt. Nevertheless, the adjustability of PT banding could favor a balanced interatrial shunt in patients with TGA, thus avoiding the Blalock-Taussig shunt. Furthermore, the device could be disinsufflated at any time if serious hypoxia occurs.

TABLE 4. Weight measurements of cardiac muscle mass indexed to body weight (in grams per kilogram)

Animals	*RV*	*IVS*	*LV*
Control	0.99 ± 0.17	1.24 ± 0.22	1.68 ± 0.35
Trained	1.72 ± 0.24	1.32 ± 0.20	1.69 ± 0.16
P value	.0001	NS	NS

Values are presented as means ± SD (control group, n = 9; trained group [subjected to 96 hours of systolic overload of the RV], n = 7) *IVS*, Interventricular septum; *NS*, not significant.

TABLE 5. Cardiac myocyte perimeter and area measurements before and after the RV systolic overload protocol (Quantimet-Leica image analysis system; original magnification 400×; n = 7)

RV systolic overload	*RV myocyte perimeter (mm)*	*RV myocyte area (mm)*
Baseline	45.9 ± 4.5	132.2 ± 25.4
96 h	58.3 ± 9.3	223.5 ± 72.1
P value	<.0001	<.0001

Values are presented as means ± SD.

Historical Notes

Some of the historical aspects conceptually related to our prototype are described here. The idea of an adjustable banding device composed of a hydraulic cuff and a self-sealing button was first proposed in 1957. In fact, Jacobson and McAllister[14] proposed a device that consisted of a rubber cuff with a lateral opening connected to a reservoir protected by self-sealing rubber. It was used on the great vessels of dogs, aiming at a congestive heart failure model. Complications in handling the device were observed. In 1969, Bishop and Cole[10] improved Jacobson and McAllister's device by covering the cuff with silicone, with the aim of reducing local tissue reaction. They induced RV hypertrophy and congestive heart failure in a dog model. In 1972, Edmunds and associates[15] introduced 2 main changes: an external, nondeformable layer on the hydraulic cuff and use of silicone instead of rubber. However, they observed asymmetric inflation or rupture of the cuff, and leakage of the injected material prevented clinical use. In 1985, a new device made of biologically stable material (medical grade silicone) was introduced by Park and colleagues.[16] The cuff was covered with reinforced braid and coated with silicone. The self-sealing button had a silicone diaphragm that did enable repeated needle puncture, avoiding leakage through the button. The device implanted in dogs and lambs was easily and effectively adjusted. In that same year, Solis and coworkers,[17] for the first time in the literature, proposed a similar device to the previous one that was intended to prepare the subpulmonary ventricle for the 2-stage Jatene operation. Nevertheless, when the system was submitted to a high gradient pressure, as in the systemic circulation, dilation of the reservoir and the connecting tube occurred. In addition, there was a tendency of the cuff to bulge laterally under high pressure. In another study the same group improved the strength of the material by reinforcing the cuff and the connecting tube with a spiral of 4-0 silk to withstand systemic arterial pressure.[18] Again, they experienced bulging of the cuff caused by a loosening silk. In our study the gradient imposed by the cuff was steady, and such deformation was not observed because of a rigid and thick cuff outer layer, which avoided centrifugal distortion when insufflated.

Echocardiographic Aspects

In our protocol the calculated pressure gradients from Doppler velocities overestimated the directly measured gradients from implanted catheters. This situation could be compared with the Venturi effect, which allows almost complete pressure recovery after the stenotic area at the vena contracta. Our data are corroborated by the in vitro studies of Levine and associates,[19] which have demonstrated a greater poststenotic pressure recovery, measured directly with a catheter, related to the extent of the obstruction, which in turn lowers the pressure gradient. These data suggest an important advantage for Doppler gradient estimation, which uses the peak velocity and provides the

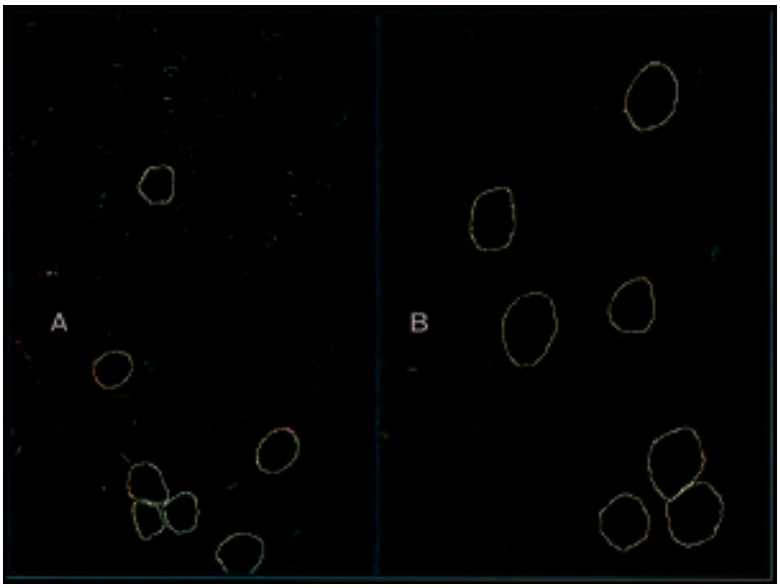

Figure 2. Microphotography of RV cardiac myocytes of animal 6 sectioned transversely at the level of the nucleus, indicating their perimeters and respective areas: *A,* baseline samplings; *B,* samplings after 96 hours of systolic overload. (Original magnification 400×.)

maximum gradient at the vena contracta, which determines the load imposed on the proximal chamber.

Morphologic Aspects

The important increase in the overloaded RV weight could reflect an increase in water content of the myocardium. Under microscopic analysis, however, there was no evidence of significant edema or interstitial fibrosis. Studies by Katayama and coworkers[20] showed that the increase in the ventricular weight subjected to a 4-day period of intermittent systolic overload was associated with an increase in the dry weight of the myocardium, relating such findings to hypertrophy of the cardiac myocyte rather than edema. However, the magnitude of hypertrophy needed for the Jatene operation remains unclear. Regarding the microscopic analysis, the myocyte perimeter and area measurements at the level of the nucleus are based on the studies of Anversa and colleagues.[21] These authors analyzed cardiac myocytes of rats subjected to 20 hours of constriction of the abdominal aorta. It was noted that in addition to the increase in the protein synthesis in the myocytes, there was a 20% increase in their area. During a longer period (13 days) of similar systolic overload, those authors found an increase of more than 200% in the average cross-sectional area of the cardiac myocytes of rats.[22] Data obtained in our laboratory[23] using a balloon catheter to induce the RV hypertrophy of young goats for a variable period of 9 to 20 days showed a 20% increase in the diameter (not the area) of the cardiac myocytes. In the present study the 96 hours of RV systolic overload induced a 69% increase in the myocyte area, which was enough to generate systemic pressures in the right ventricle. Actually, the ideal time and way of preparing the subpulmonary ventricle for the 2-stage Jatene operation remains controversial. There is evidence that nongradual and abrupt banding, causing sudden and acute systolic overload, could induce significant damage to the myocardium. A subpulmonary ventricle training program in a fashion tailored to the ventricle response should be established similarly to a fitness program tailored to an athlete, as mentioned by Redington,[24] thus avoiding the pathologic hypertrophy. Therefore an adjustable banding device would be advantageous for optimal ventricular training.

Implications

In our protocol a 0.7 RV/LV pressure ratio, a maximum 10% decrease in systemic systolic pressure, or both were used as parameters for device inflation. Other authors have used subjective criteria, such as animal clinical condition or signs of RV failure. Clinically, other parameters to indicate primary correction of TGA include a systolic subpulmonary ventricular pressure of greater than 65 mm Hg[25] or an LV/RV systolic pressure ratio of greater than 0.75,[26] although Däbritz and associates[27] have performed primary Jatene operations in a series of 7 patients aged 4 weeks or older with a pulmonary/systemic pressure ratio of 0.2 to 0.5 after submitting a trial of PT banding to systemic pressure for 15 to 30 minutes. Previous studies from our laboratory have demonstrated an equalization of ventricular thickness over a short interval of 6 to 10 days of more gradual balloon inflation[24] compared with the 96-hour period of the systolic

overload of the present study. It is noteworthy that some of the animals had systemic pressure in the subpulmonary ventricle with only 48 hours of systolic overload. However, Boutin and coworkers[5] associate the late LV dysfunction with an extremely acute overload in patients undergoing the 2-stage Jatene operation. Such dysfunction was inversely proportional to more rapid hypertrophy and to more serious dysfunction after PT banding. On the other hand, patients who present with systemic ventricular failure in corrected TGA or after failed atrial baffle operations almost always have associated tricuspid regurgitation. It is not clear whether this association is a cause or a consequence of ventricular failure. Different groups have demonstrated clinical recovery of RV function and resolution of tricuspid regurgitation after the 2-stage Jatene operation as an alternative to cardiac transplantation.[12,13,28,29] However, reoperations to tighten or loosen the band were necessary in some cases to prepare the left ventricle to tolerate systemic pressure. We believe that the adjustability of the hydraulic cuff might be more effective in training the subpulmonary ventricle in a shorter time, not only by avoiding reoperations for band adjustment but also by allowing the fine tuning of the systolic overload over that ventricle in response to a patient's hemodynamic status. Perhaps our model of variable and progressive systolic overload could favor a healthier hypertrophy of the subpulmonary ventricle when compared with the conventional stationary banding, in which the systolic stress is abrupt and constant. The gradual increase of the RV overload allowed not only progressive tolerance to the PT banding but also generated pressures even greater than that of the left ventricle after the training period. Future studies using molecular biology as a tool to assess markers of pathologic hypertrophy might compare several training programs for the subpulmonary ventricle. The main goal would be minimum cellular damage and maximum efficiency of the pulmonary banding.

Conclusion

The device proposed in this article is biocompatible and easily implanted. It was efficient in performing the adjustable PT banding percutaneously. The systolic overload imposed by the device enabled the rapid hypertrophy (96 hours) of the subpulmonary ventricles of the animals studied. This treatment might allow the preparation of the subpulmonary ventricle for the 2-stage Jatene operation not only in patients with TGA beyond the neonatal period but also in those who present with systemic ventricular failure in corrected TGA or after failed atrial baffle operations.

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